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Time for a policy on scientists' jobs

SHIRLEY Williams, when she was Secretary of State for Education and Science, was apt to complain that there was no vocal lobby for science, although there was for everything else from aardvarks to zither players. Well, that is about to be rectified when next Tuesday representatives of the Association for Researchers in Medical Science (ARMS) lobby the House of Commons about increasing unemployment among scientists.

Anne Simmonds, the convenor of ARMS, in an interview this week (p7) explains that she herself never expected to be redundant when she set up the organisation; she is a widely respected biochemist whose case underlines the difficulties and inconsistencies of assuming that in a time of cuts the "truly able" (in the words of the Medical Research Council) will find appropriate scientific employment. Moreover, it is misleading for the MRC to claim, as one official told *Nature*, that short term contract workers "are not geniuses". While this is no doubt the case (for most), it draws attention from another unpalatable but undeniable fact that many of the tenured positions filled during the boom years of the late fifties and early sixties are occupied by people less able than many in the queue behind them.

But this is merely to pose the problem: a policy of the "truly able" is one which fudges the figures to match the facts; the number of "truly able" is by definition the number who get jobs; the argument is circular and it is no policy at all.

What is needed, and urgently, is some hard thinking about the nature of scientific careers and what government can do to make proper use of this absolutely central innovative resource. It is utter folly for a government bent on establishing economic recovery to waste its scientific resources — its scientists — through a *laissez faire* policy which both wrecks individual careers and discourages

those rising on the ladder. The Science Research Council, which is responsible for most basic research in the UK, has some forward looking policies, particularly on the relation of science to industry, which must be encouraged; and the Medical Research Council which appears to be relatively backward in this regard would do well to follow suit. But some central government initiatives are required to coordinate these efforts within a broad demographic policy for science and engineering. Here is a case where science is too important to be left to the scientists, a case where the Prime Minister should exercise her stated aim of coordinating science policy where that is necessary (see *Nature* 27 September p249).

One particular matter to which she might pay attention is the role of research students, which is the engine of production of the "excess" of short-term contract workers. The pattern of using these students as a form of cheap labour for adding to a researcher's list of publications arose during the expansion of the universities, when it was virtually guaranteed that this apprenticeship would lead to a university position. This pattern continues, although the job at the end is no longer there. Breaking the pattern by greater selectivity in appointments to studentships (perhaps by examination) carries with it the further problem of what substitute technical assistance can be provided for existing researchers, and at what cost; and how to place those graduates who would at present go on to research. But this would bring the 'jobs problem' in a scientific career back to the age of 21, where it is considerably more tractable than at 24 after a PhD, or even 30 and beyond after a series of short-term contracts. We recommend to you, Prime Minister, that you ponder these matters, to the benefit not only of science, but also of the British economy. □

Soviet germ disaster shock?

SPECULATIONS on possible Soviet research into bacteriological and chemical weapons were revived this week by *Now!*, Britain's recently-founded answer to *Newsweek*. A two-page article, entitled "The Great Russian Germ War Disaster", gives a circumstantial account of an accident at a factory in Novosibirsk, last June, shortly after which, persons living in the vicinity "started to go down with a mysterious illness". "Thousands" of people, it is said, were affected, with the death rate among the victims "very high". The bodies of those who succumbed, it is reported, were delivered to the relatives in "sealed coffins". When a few relatives succeeded in examining the bodies, they were found to be covered in "brown patches".

The story, *Now!* makes clear, was nowhere reported in the Soviet press, but was transmitted to *Now!* by a "traveller". It should be recalled, however, that except for international air-crashes or accidents involving foreigners, it is very rare for the Soviet press to report accidents. Certainly, to judge from the *Now!* reports, something untoward happened in the Novosibirsk factory — but the account, as *Now!* itself admits, is consonant with a Séveso-type chemical disaster, which need not necessarily have any connection with the military at all. The allegation of

bacteriological warfare seems to be based on the fact that a molecular biology institute was established at Novosibirsk some three years ago.

Certainly, the now famous Kyshtym nuclear devastation of 1958 is a constant reminder that the Soviet Union has the logistic capacity to mount a major cover-up operation, even to the extent of evacuating large tracts of country. Moreover, in conditions of a controlled press, even the most innocent natural disaster is liable to generate wild rumours. (Last winter's explosion, which wrecked the Rotunda building in Warsaw, was attributed, in popular belief, to a bomb planted by the authorities . . . so that the dissident "Committee for Social Self-Defence" issued an appeal to the government to allay panic by publishing the result of the commissions of enquiry).

The official Soviet News Agency TASS always vehemently denies any rumours of bacteriological weapons research. Nevertheless, if what happened at Novosibirsk had no military applications, it is perhaps unfortunate that, immediately following the *Now!* allegations, the Soviet press attache in London was away on sick leave, leaving no deputy empowered to comment. □



Can Yankee ingenuity keep US physics on the ball?

As US high energy physicists bemoan a continued tightness of funds, West Coast scientists are proposing an ingenious way of keeping up with European colleagues — at a fraction of the cost. **David Dickson** reports

Inflation is threatening to upset the fragile truce agreed two years ago between the US research community and the Office of Management and Budget over the appropriate level of support for high energy physics.

The agreement negotiated by the Department of Energy guarantees a constant 'floor' of support (adjusted for inflation) for research at the three main accelerator laboratories — the Fermi National Laboratory, the Brookhaven National Laboratory, and the Stanford Linear Accelerator Center — pegged at the 1979 level of \$300 million a year; but it promises no more, unless a particularly good case can be argued.

Initially physicists accepted the agreement since although it means less money than they would have liked, at least it provided medium-term security. But now a higher-than-anticipated inflation rate means that the physics budget is falling considerably behind steady-state funding, perhaps by \$30 million over the next two years; and this is having serious implications for current and projected research programmes.

It also increases uncertainty over whether Congress can be persuaded to support two projects which would enable US physicists to keep up with their European counterparts as the latter pursue the intermediate vector boson (Z^0) predicted by the Nobel-prize-winning theories of Steven Weinberg, Abdus Salam and Sheldon Glashow.

The first is a new facility proposed by Fermilab to help investigate the proton-antiproton collisions that should produce the Z^0 ; the second is an ingenious plan devised by Dr Burton Richter of SLAC to study the physics of the Z^0 and its decay particles at a fraction of the cost of Europe's proposed 100 GeV electron-positron collider, LEP.

Compared to the early 1970s, the current picture for US high energy physics is far from gloomy. Indeed from one point of view the community is doing very well, with major construction projects under way at the three main laboratories, as well as Cornell University. "The current programme is basically sound, and there has certainly been a reversal in prospects since 1975" says Dr Tony Samios of Brookhaven.

There are, admittedly, some slight hitches. At Brookhaven, Long Island, for example, designers working on ISABELLE, the 400 GeV intersecting

proton storage rings, are having difficulty in getting the superconducting magnets to produce the required magnetic field; it's a problem which has led to "particular agony over the past few months", says project director Dr Jim Sanford, though he remains confident that it can be solved by the time the magnets should be ready for mass production next spring.

Elsewhere the picture is brighter. Fermilab near Chicago claims to be up to schedule with its own production of superconducting magnets, designed to double the accelerator's energy from 500 to 1000 GeV; SLAC, in California, injected its first positron beam into its positron-electron collider PEP last week-end, and is planning to start operating on schedule — and within the \$78 million budget — in late January 1980.

The biggest cloud around is rising inflation. The DoE's budget allocation for the current fiscal year allows for 7% inflation. In real terms, inflation in the US is well into double figures; and construction costs on new facilities have increased even more. The projected 1981 budget of around \$350 million could be about \$20 million less in real terms than the 1979 level previously promised.

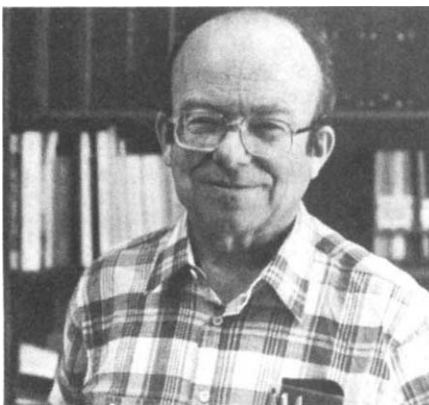
Of particular concern are the high electricity costs caused by oil price increases, a factor to which accelerators, with their vast power consumption, are particularly vulnerable. Brookhaven, for example, has recently been faced with an almost 50% increase in its power rates; and at SLAC, the extra power needed by PEP will require shifting to a new source of supply at much less preferential rates, which will increase the laboratory's annual power bill from \$2 million to almost \$5 million when PEP is fully operational.

Since salaries are fixed, the extra costs will have to be absorbed by operating budgets. The result could be a significant curtailment of experiments. SLAC is already warning that when PEP comes on line, it will have to operate considerably below capacity; and Dr Leon Ledermann, Fermilab director, is talking of having to close down a complete experimental facility, the 15-foot bubble chamber used for neutrino-beam experiments, if additional support is not forthcoming.

Up to now, physicists have told the DoE they were confident a viable national programme could be maintained at the three accelerators on the \$300 million figure. "Now either the policies have to be changed or the funding has to be changed;



Ledermann of Fermilab: experiments may have to close



Panofsky of SLAC: "change the policies or change the funding"

we cannot pay lip service to both", says Dr Wolfgang Panofsky, director of SLAC.

What makes the situation particularly frustrating is that US physicists see themselves having to sit back while their European colleagues use new facilities such as the super proton-synchrotron (SPS) at the European Centre for Nuclear Research (CERN) and the proposed LEP, explore the new areas of physics that will be opened up if the existence of the Z^0 particle is confirmed.

The two proposals now on the drawing board for keeping up with these developments would therefore fill a major gap that could develop within the US high energy physics programme. Fermilab's plan is to build a new machine which will help physicists to study the proton-antiproton collisions expected to produce the Z^0 .

The plan requires a pre-booster storage ring, which would collect anti-protons created by protons extracted from Fermilab's main ring, and cool them sufficiently to reinject them in the opposite direction to the protons. The machine would come on line after PEP, which is expected to be the first to discover the Z^0 (if

it exists) soon after coming into operation in 1981 (perhaps). It would produce a considerably higher collision rate than the equivalent European machine; and Fermilab physicists are hoping that this will help persuade Congress to provide the necessary \$26 to \$29 million.

The second proposal, devised by Dr Richter, is to create single collisions between individual pulses of electrons and positrons. Both would be produced on the SLAC linear accelerator, using techniques now being developed to increase the beam energy from around 25 GeV to 50 GeV; they would then be made to circulate in opposite directions around a circular tunnel, and carefully aimed to collide with each other.

Since each pulse will only produce a single collision, the event rate will be much lower than for the constantly circulating pulses in storage rings at a comparable energy. Dr Richter proposes to make up for this, however, by tuning the two beams to collide at a centre of mass energy calculated to be equal to be the mass of the Z^0 particle, using the resonance at this energy to increase the luminosity.

If the Z^0 does exist, then the Single Pass Collider, as the project is known, would become a virtual factory of decay particles with an event-rate around 10^6 a year, and the facility could be used by many different users. If the Z^0 does not exist, the event rate will be very much slower; "but the physics will be much more interesting, since it would mean a basic flaw in the theory", says Dr Richter.

The design would lack the power and versatility of LEP, scheduled to reach 100 GeV per beam, and to be built by the late 1980s. But at a cost of \$60 million — compared to LEP's \$1000 million — plus the fact that it could be in operation as early as 1984, the proposal is attractive to US physicists. "It is an extremely clever idea, real 'Yankee ingenuity' " says one.

It all comes down to money. Dr Richter has asked the DoE for \$800,000 to carry out a preliminary design study; and says that, if given the go-ahead by the department and Congress in the 1982 fiscal year budget to be prepared next summer, the collider could be completed in under three years.

Whether Congress will go along with either this or the Fermilab project remains the great unknown; and few are over-optimistic. Last week the DoE's High Energy Physics Advisory Panel (HEPAP) supported a proposal that its chairman, Dr Sidney Drell of SLAC, write a letter to the department expressing grave concern at the current funding position; and warning that if there was not an immediate infusion of extra funds — at least sufficient to restore the budget to the promised "floor" — the panel would consider withdrawing its endorsement of the national programme. Department officials at the meeting promised the physicists a sympathetic hearing; but no more than that. □

NIH director unlikely to grant exemption from controls for DNA experiments

The director of the US National Institutes of Health, Dr Donald Fredrickson, seems unlikely to accept in full the recommendation of the Recombinant DNA Advisory Committee (RAC) that a large body of recombinant DNA experiments be exempted from the guidelines established by the NIH, following widespread criticism of the implications of such a move.

Sources within the NIH say that Dr Fredrickson will probably approve a significant reduction in the safety precautions needed to carry out most experiments using as host the disabled K-12 strain of the bacterium *Escherichia coli*; these are said to account for between 80 and 85% of all experiments using recombinant DNA techniques.

But whereas the RAC, in a split 10 to 4 vote, decided at its last meeting in September to recommend exemption for the guidelines for such experiments — with merely the ruling that they should be registered with local biohazard committees, and should preferably be carried out at the minimal PI physical containment level — Dr Fredrickson is likely to insist that the PI conditions be enforced. This will include strict adherence to technician training requirements, and a ban on practices such as mouth-pipetting.

The NIH has had a flood of comments since the proposal to exempt the experiments was first put forward by RAC members Dr Allen Campbell and Dr Wallace Rowe at the committee's meeting in May. Many scientists wrote supporting the proposal, including in particular a petition signed by 183 scientists attending the Gordon conferences on nucleic acids and on biological regulatory mechanisms.

Others, however, have expressed concern about the implications of such a drastic move. Professor Roy Curtiss, of the department of microbiology at the University of Alabama in Birmingham, wrote to Dr Fredrickson criticising the proposed exemptions as premature, given

the uncertainties that still exist over the hazards of recombinant organisms. "I believe that the RAC's recommendation to you was based more on the politics of science than on its data", he wrote.

In addition, the Natural Resources Defense Council in New York has demanded that an environmental impact statement be carried out for the proposed safeguard reductions, arguing that "the proposed exemptions are of such breadth and importance as to require full compliance with the National Environmental Policy Act."

In view of the controversy surrounding the committee's recommendation and its implications, Dr Fredrickson has been carrying out a detailed review both of the arguments used during the RAC meeting, and of the data used to support them.

The results of this review will not be known for several weeks. However it is thought that Dr Fredrickson is reluctant to face the criticism that total exemption would involve.

For example, two weeks ago the citizens of Amherst, which has introduced a local ordinance requiring recombinant DNA research at the University of Amherst to be conducted under the NIH guidelines, agreed to require adherence to the 1978 revision of the guidelines, rather than their stricter 1976 original version.

However according to Dr Bruce Levin, a research scientist at the university who has been closely involved in the local debates, there would probably be strong resistance from the local community if a large proportion of the experiments were to be exempt from regulation.

Dr Fredrickson, however, is said to be prepared to accept most of the arguments in favour of a substantial reduction in required containment levels for many experiments involving the K-12 strain; one of the few areas still to be resolved is whether local committees would be required to give prior approval to experiments using biologically active materials, such as active polypeptides or active proteins.

The NIH is paying particular attention in its review to uncertainties that have arisen as a result of various risk assessment experiments, such as those which indicate that bacteria into which plasmids have been introduced can survive considerably longer than expected in the human gut.

Meanwhile staff members for Senator Adlai Stevenson's science and technology subcommittee are preparing legislation that would require all non-federally supported research involving recombinant DNA techniques — in particular that carried out by private companies — to register their experiments with the NIH. At present companies can register; but the arrangement is voluntary. □



Fredrickson: reviewing the arguments

Pollution control: how an EEC directive muddled the waters

Britain and the EEC have been arguing for many years about the best way of controlling pollution. The conflict arose not because anyone disagreed that certain substances should be controlled but because the EEC wanted to lay down a blanket standard of emission into rivers from sewers and factories.

Britain argued that this was wasteful and inefficient because it took no account of the use of a particular river nor its capacity to dilute and disperse waste materials.

Britain wanted local controls, based on local conditions and local usage, as long as there was no risk to human health or animal life.

Eventually, in December 1975, the EEC Council of Ministers compromised and agreed to let the British control pollution in their own way. But now new directives have been issued from Brussels concerning the control of specific substances in water and they put a question mark over that 1975 agreement

Extracts from a speech by Lord Ashby (right) to the House of Lords in London last week

The first of the 'drin' Directives sets a limit for the emission of dieldrin. It is 60 parts per billion in the waste water discharged into the river. Clearly, the Commission considers that this is going to be a little difficult because it allows factories time to build treatment plant. The limit is not to become binding until 1986, six years ahead. The second Directive offers the other option, the one we asked for: an environmental quality objective for the river into which the wastes are discharged. The level proposed for this is 0.005 parts per billion. Unlike the first option, this level has to be reached in two years' time, which gives the impression that it is an easier option.

These requirements make a nonsense of the options agreed by the Council of Ministers after great pressure from the British Government in 1975. First, the two options are mutually contradictory and inconsistent. Suppose we complied with the first Directive. Suppose we reduced emissions to 60 parts per billion in the Calder River (the river most affected in the UK). Would this result in an environmental quality objective of 0.005 parts per billion? It certainly would not. After doing a little arithmetic, which is fully within the capacity of the staff of the Commission, it has been found that it would need a flow of one hundred times that of the Calder at its minimum value in order to produce that degree of dilution; in fact many times the flow of the Thames at Teddington.

So the two proposed standards simply do not match. This could either be a deliberate soft option for those member states that want to choose an emission standard or it is a sign that the scientific basis for these figures is very suspect. That is not the only difficulty. The standard of 0.005 parts per billion is inconsistent with two other Directives which have already been published by the Commission and agreed by the Council on their own definition of pollution. For instance, the Commission has already agreed in principle that the level of dieldrin in water to be used for human consumption is to be not more than 0.1 part per billion — that is, 20 times higher than

the level proposed in this Directive — so the limit has obviously not been set to make the water safe to drink.

What about the fish? Three years ago the Commission published its own water standards for fish. In this Directive, which has now been adopted, pesticides are not included at all. However, it is possible to calculate what the safe level will be because it is known that for a coarse fish like the roach — and we are talking about coarse river fishery in the Calder — about two parts per billion is the beginning threshold value for damage, and the level of dieldrin in the Calder varies a good deal; but it is in the region of 0.1 part per billion, that is one-twentieth of the danger level.

So on two counts, both falling in the definition of pollution by the Commission itself — human health and the health of fish — the environment quality objective proposed just does not make sense. There remains a very important fact, that the fish and other living things accumulate dieldrin from water, and they concentrate it enormously in their own tissues. If people then eat the fish they might be at danger. Experts are reluctant to commit themselves to precise figures for the amount of dieldrin which fish and other living things will accumulate from the environment. However, it is a general consensus that it is a multiple of 10,000 of the concentrations outside.

If the Calder carried trout, salmon or eels — which it does not because of pollutants other than dieldrin — then there would be an indubitable case for monitoring the dieldrin level of the fish. Then consent levels on the river could be imposed which would guarantee that the acceptable daily intake, if people ate the fish every day would be safe. This is exactly what is being done all the time in Britain in waters where fish are eaten. But coarse fish are not eaten in Britain, and the present use to which that stretch of water is put does not justify such drastic lowering of the dieldrin level either — to come back to the Commission's own definition — on grounds of hazards to human health or on grounds of harm to living resources.



Finally, there is a practical difficulty about settling a standard of 0.005 parts per billion which the drafters of the Directive do not seem to have noted. The waters which fall into a river from a mothproofing plant such as those on the river Calder (these plants produce the dieldrin) contain many impurities. If one wants to analyse the water containing those impurities for the concentrations of dieldrin, one simply cannot detect levels lower than about 0.01 parts per billion, so the level proposed in the Directive is one which could not in fact be measured, except by most refined and difficult research techniques. So the idea of monitoring concentrations of that level as a routine matter simply is not on.

These are the reason why I think it is not an exaggeration to say that "nonsense" is the word that has to be used for this option offered to the nation states in the Community which prefer to take environmental quality objectives rather than emission standards. How did the Commission come across this figure? The Directive states that the figure was reached "after consulting a group of national experts", and also on the basis of a study made by consultants in the Agricultural University of Wageningen in Holland. But as with previous Directives about the environment, scientific evidence for the decision is not given and the consultants' report is not published.

However, I have talked with the British experts in this matter and their view is clear from what I have already explained to your Lordships. I have managed to see a copy of the consultants' report. Again for the record, I should like to read the conclusion of that report: "This leads to the rather disappointing conclusion that most toxicological studies made so far with the aim to assess the environmental hazards of aldrin, dieldrin and endrin . . . have been inadequate in the sense that for the aquatic environment no practical environmental threshold levels can be derived from their results".

The Commission then concludes, based on that conclusion, without providing any of the consultants' data, that the

environmental quality should be set at a level which is inconsistent with two other Commission Directives, unjustified on grounds of safety to human beings or wildlife, and below the limits detectable by routine analyses. As one of our witnesses put it, data appeared to have been plucked out of the air using a concentration factor much higher than the one thought to be reasonable by British experts.

We all want to reach amicable agreement with the Commission over an environmental policy for the Community, and we all agree that dieldrin is a harmful substance which ought to be phased out as soon as satisfactory alternatives are found. But we see no prospect of amicable agreement unless the Commission can be persuaded to adopt two simple principles when it prepares its Directives.

●The first principle is to abide by a declaration of the Council of Ministers made in 1973 about the environment. This declaration is the only authority for having a European environmental policy at all. It states that protection of the environment should be achieved "at the lowest cost to the Community" and that in setting quality objectives "proper account must be taken of the specific characteristics of the regions in question".

This means the deployment of our limited resources in places where they could be most cost-effective. It means that we should have different environmental qualities for rivers, depending on whether they carry game fish, coarse fish or no fish at all. Following this policy in Britain over the last 20 years, we have increased the amount of unpolluted rivers by about 4,500 kilometres and we have diminished the amount of badly polluted rivers by about 1,500 kilometres. I doubt whether any other member state in the Community can match that record. A doctrinaire pursuit of uniform emission standards would wreck this policy.

●The second principle is to insist on free and candid disclosure of the scientific evidence on which Directives are based. It simply is not good enough for the Commission to say, "After consulting a group of experts, we set such and such standards". The Select Committee has now got plenty of evidence that the experts' advice is not always taken. To comply with some of these Directives — for instance, that proposed for fish — would put member states to enormous expense and to expect them to incur this enormous expense without even seeing the data on which the standards are based is, I suggest, an intolerable situation. I am afraid it is true — and we regret it — that there will be perpetual wrangling over environmental policy in the Community until this freedom of information is secured.

Compared with the Common Agricultural Policy, the Commission's environmental policy for Europe may well be a small matter. But it is a perpetual source of irritation.

UK to tighten asbestos controls

TIGHTER safety standards for asbestos are proposed in a report published last week by the UK Health and Safety Commission (HSC). The report, prepared by the commission's Advisory Commission of Asbestos (ACA) makes proposals, which according to the committee's chairman Mr Bill Simpson would, if they became law, "mean that Britain would share with Sweden the most stringent asbestos rules of any country".

The report recommends that the standards currently in operation regulating asbestos fibre concentration should be halved to 1 fb ml⁻¹ for chrysotile (white asbestos); reduced four-fold to 0.5 fb ml⁻¹ for amosite (brown); and that a statutory ban be placed on crocidolite (blue) in the UK. In addition, until such fibres as crocidolite are replaced, the standard for its removal should remain at the present 0.2 fb ml⁻¹.

It is proposed that the new standards should take effect from 1 December 1980 and that in future they should be referred to as "control limits" rather than "hygiene standards", the latter definition suggesting an asbestos concentration below which exposure is safe.

Simpson firmly believes that the new standards will reduce the incidents of asbestos-related diseases in future. It is the report's view that exposure to one fibre per mille of chrysotile daily over a period of 50 years will cause an increased mortality rate of 0.02 to 1.25 per cent. This prediction is based on the results of the only three surveys — two North American, one British — which provided adequate dose/response data. Calculations of mortality rates based on these results have their problems, however, as the report readily admits. Much of the data in these surveys on asbestos concentration was obtained by measuring particle counts, a far less precise method than the normal practice of counting fibres.

As far as the public is concerned, the report concludes that there is no quantitative evidence of risk from over-exposure to asbestos dust. The report says that despite the fact that there is a high proportion of individuals, especially in urban areas, with asbestos fibres in their lungs, there has been no general reporting of asbestosis. Accordingly the report suggests that "there may be a threshold level" below which asbestosis is not detectable.

This, however, does not hold true for the carcinogenic properties of asbestos. The report states baldly that there is "no evidence" for the existence of a threshold below which lung cancer or mesothelioma — of the pleura or the peritoneum — will not occur. It is the report's view, however, that crocidolite is consistently more dangerous than chrysotile and should be treated accordingly. Swedish and Dutch

regulatory authorities agree.

The report acknowledges that all forms of asbestos will cause lung cancer. In numerical terms, lung cancer is the great killer but the report says "included in this excess mortality from lung cancer is a substantial element due to the synergistic action of tobacco with asbestos, which cannot therefore properly be attributed to the effect of asbestos alone". The asbestos industry has always made much of this synergism to play down the role of asbestos in the aetiology of lung cancer.

On the question of death due to mesothelioma, the report is unequivocal; the majority of these are due to exposure by chrysotile. Death from mesothelioma reported in American asbestos workers (the report says) are attributable to exposure to both amosite and chrysotile and not to chrysotile alone.

Deaths from mesothelioma in the UK doubled between 1967 and 1976 — currently standing at some 320 per annum — and the report expects the number to increase before the peak is reached.

The asbestos industry makes no pretence of being pleased about the December 1980 deadline for enforcing the 0.5 fibre per mille limit for amosite. Mr S D Hardie of Turner and Newall and an advisory committee member says that the deadline is too tight. He insists that working to 0.5 fibres per mille is pushing the technology to its limit. The industry, he says, can work to one fibre per mille of chrysotile but, as far as the 0.5 fibre per mille for amosite is concerned, Hardie says he could not "put his hand on his heart and say to the work force that it is not being exceeded".

Criticism that the advisory committee has been too sensitive to the needs of industry has been voiced by the Society for the Prevention of Asbestos and Industrial Diseases. Mr Max Madden, a trustee of the society and former Labour MP for Sowerby — a constituency which includes the ACRE Mill asbestos factory at Hebden Bridge — was frank about the pressure on the advisory committee. In his view, the committee had been subjected to considerable propaganda from the industry and some of it had succeeded. Anticipating criticism of its report, ACA chairman Bill Simpson emphasised that the control limits are not a "once and for all" exercise. New evidence will be constantly reviewed, and every encouragement was being given to the development of safe asbestos substitutes.

Comments on the report are invited and should be submitted by 31 January 1980.

Alastair Hay

Asbestos Volume 1, final report of the Advisory Committee, £5 (p + p).

Volume 2, papers prepared by the Advisory Committee £5 (p + p) available from HMSO.

news in brief

\$40 billion law suit over Dioxin damage: A veteran of the Vietnam War is suing five US chemical companies for \$40 billion damages, claiming that he suffered from brain cancer as a result of exposure to the defoliant 2, 4, 5-T, known as Agent Orange. The defoliant was used extensively by the US in Vietnam between 1962 and 1971, and recent studies have indicated that its use as a domestic herbicide may be associated with foetal defects in pregnant women. According to the man's attorney, the purpose of the suit is "to establish a trust fund to help the veterans who discover they have been poisoned after the statute of limitation runs out, and for the children yet unborn who will have birth defects".

Waste disposal problems halt radioactive research: Research workers at Harvard University have been told that research involving the use of radioactive tracers will have to be stopped because of difficulties in the disposal of radioactive waste. This follows the closure last week of the US's two main dumping sites for liquid low-level radioactive waste, Hanford in Washington state, and Beatty in Nevada. A third dumping site, Barnwell in South Carolina, refused to accept such wastes last spring.

Many major laboratories and hospitals are now faced with mounting accumulations of liquid waste which will have to be stored until alternative disposal arrangements can be made. The government is considering creating new temporary disposal sites or using the six national laboratories operated by the Department of Energy as temporary dumps. Another alternative could be the incineration or evaporation of the waste products. NIH disposes of its absorbed wastes in this fashion under an agreement with Todd Shipyards in Galveston, Texas. According to Dr Mortimer Litt of Harvard, an insignificant amount of radiation is released by this process, well below the exposure standards established by the Nuclear Regulatory Commission, and would not constitute a public health risk. — *Anne Norman*.

Israeli drought threatens Dead Sea: Israel faces water rationing by next summer, unless there is an exceptionally wet winter to replenish the Sea of Galilee — the main reservoir of the country — now at an all-time low. This year's severe drought will not only have a severe effect on the country's agriculture and industry, it has also caused severe damage to the ecology of the Dead Sea, which gets 65% of its water from the Sea of Galilee via the lower Jordan. Several Israeli scientists are demanding urgent government action on the long-discussed proposal to construct a canal from the Mediterranean to the Dead Sea. Dr Sholomo Drori, Information Officer of the Dead Sea Potash Works at S'dom, told *Nature*: "No one is putting pressure on the government for the important things, like a canal from the Mediterranean to the Dead Sea. The Southern part of the Dead Sea has vanished, probably for ever".

Professor Anthony Peranio of the Technion, renowned for his interest in renewable energy sources, said: "We should be building this canal. There should be great pressure brought to bear on the powers that be to get free-flowing water from the Mediterranean to the Dead Sea." Because the Dead Sea lies some 600 metres below the level of the Mediterranean, the canal, he said, could be harnessed for energy purposes, to generate electricity and cool inland power stations.

First French cosmonaut to be trained next year: Franco-Soviet space cooperation has taken a step forward with the announcement on 23 October that a French astronaut will be part of the crew of a Soviet spacecraft to be launched in the middle of 1982. This follows a meeting on space cooperation — the sixteenth such meeting in 30 years between France and the Soviet Union — in Corsica from 15 to 20 October. The meeting also agreed on details of a study of the atmosphere of Venus and reviewed the possibility of cooperation in biology, geophysics, meteorology and telecommunications.

"Truth in testing" legislation defeated in US: A bill requiring disclosure of the methods used by the companies which set and administer standardised educational tests has been defeated in the US Congress after an intensive lobbying effort by the testing industry. Tests such as the standard aptitude test (SAT) are set and administered by largely unregulated private firms. The tests determine the education of most American children from assignment to classes for the mentally retarded to college entrance, and are enormously influential. The so-called "truth in testing" legislation would have required (among other things) that the companies disclose examination questions. Higher education organisations and testing companies opposed the bill, claiming that disclosure would result in undue influence on school curricula. They also said it would be prohibitively expensive to prepare new questions for every exam and that the quality of questions would suffer. A proponent of the bill, Dan Cameron of the National Education Association, questioned the industry's motives. "The testing industry exerted all the influence it has to kill this legislation because they don't want to reveal what this legislation would make them reveal", he said.

● The industry suffered a defeat last week when a Federal judge ruled that standardised tests could not be used by California officials to determine the placement of black children in classes for the mentally retarded (*Nature* 25 October).

ISTC planners accused of illegal lobbying: Administration officials may have acted illegally by using funds appropriated by the US Congress to gather support from private individuals and institutions for the proposed Institute for Scientific and Technological Cooperation (ISTC), according to a report prepared by investigators for the Senate Appropriations Committee. The accusations have been passed on to the Department of Justice, and they could be the death-blow for the Institute, a centre piece of the US presentation at the United Nations Conference on Science and Technology for Development. The ISTC is now struggling desperately for congressional approval.

Three weeks ago, the US Senate agreed by 70 votes to 22 to cut all funds for the Institute out of the Foreign Aid Appropriations Bill. This was largely at the instigation of Senator Dennis Deconcini of Arizona, who earlier in the year had persuaded the Senate not to approve the ISTC's authorisation on the grounds that it would merely create another unnecessary government bureaucracy. The latter decision was later overturned in a conference with the House, and it had been hoped that the budget decision would also be reversed, since the House has already agreed to provide \$23.75 million of new funds for the Institute. However the latest findings about the ISTC's lobbying activities have not increased its chances of success.

New crack in French reactor increases hazard: A further step in the crisis of the French nuclear programme has occurred with the revelation that there has been a leak of primary coolant into the secondary steam-generation circuit at the operational nuclear reactor Bugey-2 near Lyons. This was confirmed last week by the French trade union federation, the CFDT and is exactly what was feared might happen after the discovery of cracks, at 25 of France's 26 pressurised water reactors. The cracks were in the tubular collars that are supposed to seal off the radioactive primary coolant from the water circulating in the steam-generation areas (*Nature* 27 September) and mean that large sections of the reactor are now subject to radioactive contamination. The original discovery of cracks has already led to a strike and consequent postponement of the loading of two newly-built reactors (*Nature* 11 October). This time the state-run Electricité de France has refused to shut down the Bugey plant and has declared a moratorium on all public information unless previously cleared by government authorities. — *Jim Ritter*.

Portrait of the out-of-work scientist

Government expenditure cuts are just statistics to some; to others they mean personal and professional tragedy. **Joe Schwartz** reports on those who are fighting back

Dr Anne Simmonds is an internationally known research biochemist specialising in purine metabolism. Forceful and competent, her problems as director of the Purine Laboratory of Guy's Hospital Medical School in London for the past eight years include convincing granting agencies that the study of a metabolism is as important as studies of specific diseases.

The field is a lively one. Studies of the loss of enzymatic links in the purine recycling process make specific interventions possible for at least five bizarre and life-threatening clinical abnormalities. One of these is the Lesch-Nyhan syndrome, a severe neurological disorder in children characterised by spasticity and self-mutilation which is due to a defect in one of the salvage enzymes of the purine cycle.

Simmonds' work, in conjunction with clinical colleagues, has led to her being "in the right place, at the right time, with the right expertise to be able to recognise a new disease for the first time". Her busy schedule includes the activities associated with an established international researcher: appointment to the Organising Committee for the Second and Third World Congress on Purine Metabolism in Man, invited participant from the UK to the Second World Symposium on Inborn Errors and Immune Disease in New York, and an appointment as Visiting Professor in the Department of Medicine at the University of Munich for a period early in 1980. Professor Fred Rosen of the Department of Pediatrics of Harvard Medical School describes the field as "bursting with excitement" and acknowledges Simmonds as having done "well known and very fine work with very widespread implications".

In September 1980, the Purine Laboratory will be closed and its Director, along with her staff of 4 graduates and one doctoral student will be unemployed.

Anne Simmonds is one of many senior researchers in the UK with international reputations and a long history of successful and productive research who are slowly being squeezed out of their research careers. Simmonds is vulnerable because she has moved through a succession of short-term contracts, the 'soft money' of the 60s, in order to be able to do her work. In the present period, research councils have found it convenient to regard short-term contract workers as dispensable and with 50% to 70% of the UK's basic research workers being supported by contracts researchers like Simmonds are

caught in the net.

Simmonds' case is an important counter-example to official rhetoric about the effects of cuts on research programmes. Contract workers are portrayed as 'young and adaptable' or as old and 'burned out'. Official talk about the need to 'make room for new blood' or to 'maintain excellence' has muted criticism of recent policy decisions, by making a virtue out of the perceived necessity to cut public spending. Senior researchers who are unfortunate enough to find themselves in vulnerable positions have not only to face the loss of their jobs but also attacks on their competence for no longer being 'good enough' to warrant support.

Simmonds stresses that many scientists working in research full time on contracts have voluntarily given up other permanent jobs because of their commitment to research. In her case, she has turned down three permanent clinical jobs as well as a position at the Wellcome Laboratory in Beckenham. Her refusal of the Beckenham job illustrates some of the problems facing researchers in the UK. Simmonds was hired in 1967 at the Wellcome to do purine research. But in 1970 Wellcome decided to transfer most of its purine programme to the US. Simmonds was offered a chance to go on to Wellcome's permanent staff but after accepting she found that the UK purine programme was being eliminated and that she would be required to work in immunology instead.

Through the intervention of Dr David Long, then Director of the Wellcome, and with funds provided, in true 19th century style, by an anonymous donor, Simmonds was able to set up a purine lab at Guy's in 1971. Her support from Wellcome stopped in 1972. She has since received a 3-year non-renewable contract from the MRC with the MRC making it "very plain" that they were not happy about a person of "advanced age (43 at the time) not having a permanent position." She was able to get a final three year bringing grant from the Wellcome in 1977 and with its termination in 1980 the work at Guy's will stop.

Simmonds has applied to "every conceivable" charity for funding from

EEC funding agencies to the Cadbury, the Sainsbury and the Kellogg foundations. She has not reapplied to the MRC. "If I was too old for them then, I am too old for them now. The MRC has always been less than helpful and we have either been ignored or forced to listen to insults about our capacity to research." As Simmonds puts it: "Because we have found it impossible to continue to do our work well under current pressures, we have dared to question our traditional role — and we have rocked the boat".

The MRC says that their records show that Simmonds had applied for and received a normal three year non-renewable grant in 1974 and has not applied since then. "The MRC has no concerns in this particular situation. She can apply for a grant as can everyone else and whether she does or not is entirely up to her. This is a matter for Guy's Hospital, not us", an MRC official told *Nature*. The loss of Simmonds lab means that there will be no work of international stature on purine metabolism in the UK.

Simmonds has had the energy and commitment to fight these pressures organisationally. She is one of the founding members of ARMS (Association of Researchers in Medical Science) established in June 1978. "I was originally concerned because of the general problems facing staff: short term contracts, the loss of two years in every five or six writing and rewriting applications and the loss of younger people just when they have learned enough to be useful."

ARMS now has branches in both London and Manchester. It is attempting to convince UK authorities that a career structure for non-medically qualified full time researchers is a long overdue necessity for the health of British medical research.

Simmonds describes her involvement in ARMS' campaign in personal terms: "Two of my senior colleagues with outstanding records, who, when ARMS was set up last year had 12 months left, are now out of work. This had happened despite the fact that they have actively contested their abandonment by the MRC after 13 years of short term support, both to that body and



Up in ARMS: committee members Sue Barlow, Mick Kadlubowski, Anne Simmonds, Arlene Unger

to local authorities. Today I am in exactly the same position. I have one year left and my staff will have to follow me into the street since the school will not support them beyond the termination of my own grant." Simmonds intends to devote her political energies in the coming year to collecting data on the current treatment of

senior scientists like herself. As far as her research goes she is more philosophical. "Although it is difficult to believe that there is no official recognition when one has done something as clinically useful as describing a new disease, we have to accept that we are in the wrong field with the wrong qualifications." □

Where MRC stands on jobs

THE Medical Research Council's current career structure for non-clinical scientific staff was agreed with the Association of University Teachers and came into force in 1974. It applies only to staff in the MRC's units and provides for three categories of appointment: three-year short term contracts, five year limited term appointments and tenured posts.

In general a new PhD would apply first for a three-year short term contract. At the age of about 27, after completing one three year term, he/she would then be eligible to apply for a five year limited term appointment. At the end of that, at age about 31, he/she can apply for tenure.

The five year limited term appointment was created as a bridge between post-doctoral appointments supported by short term grants and tenured posts. The idea was that 75% of those with limited term appointments would receive tenure at the end of five years. Those who did not, would not normally be eligible for further three or five year appointments. This scheme was designed to prevent researchers continuing work on limited term grants into middle age by forcing them into a career decision in their early thirties.

The MRC currently employs 790 non-clinical scientific staff in its units. 21% of them are on short term contracts, 14% on limited term and 65% have unlimited tenure. The target figures, which the Council would hope to achieve after the system had been working for several years, are 18%, 15% and 67% respectively. However, with pressure on budgets due to public expenditure cuts, the MRC may never achieve these figures. It has recently been rethinking the career structure for non-clinical scientific staff and has put forward a proposal to the AUT for negotiation, which is likely to include a major modification of the tenure system.

The position in the universities is very different. The MRC provides tenured staff with grants for research projects. Untenured staff are paid out of these grants, the university being their employer. The MRC currently provides funds for 800-900 research studentships and 1300-1400 scientists on short term contracts. Half of the latter have PhDs and half have only first degrees.

Seven years ago, the MRC began to realise the problem of absorbing all these short term contract workers into permanent positions. It has, however, according to one official been surprised at

how slow the crisis has been in coming. Many people have left the system by taking jobs overseas or moving right out of research. "The problem (of short term contract workers) will solve itself by the scientists making an appreciation themselves and then making the necessary adjustments". However, the Council does not know how far the process of adaptation has gone and criticises the AUT's attempts to quantify the problems. "The (AUT's) figure of 2500 unemployed scientists hasn't been substantiated and we don't really know if there is a problem, whether people have been able to adapt and whether able people have been discouraged from entering research".

The MRC's main response to the lack of tenured posts has been to create bridging fellowships to enable the "truly able" post-doctoral worker to wait longer than the normal five year limited term for the right tenured position to turn up. But the scheme has met with minimal response. The Council has also resisted pressure from within its own ranks and outside to recruit more graduate students.

Ideally, the MRC would like to have more control over research posts. Two MRC officials at least would like to judge the "hallowedness and timeliness" of research and to see a "population of mobile research workers who could move around at the Council's discretion". Another, Tony Vickers, told a meeting at the Royal Society of Medicine earlier this year that "it might not be a bad idea if we had to revert to immediate post-war conditions". This would mean a much smaller scale of scientific activity with no post-doctoral researchers. "Serendipity goes out of the window when postdoctoral workers come in".

The Association of Scientific, Technical and Managerial Staffs has been trying for several years to gain 50% membership on the MRC. Its attempts, however, have been resisted. It just wants "to turn Council into a private benefit society" according to one MRC official. "The Council is a self-perpetuating oligarchy" which has trust in its members who in turn have trust in it.

ASTMS, however, takes a different view. It considers fixed term contracts as a "primitive way of dealing with scientific and technical staff" which places "too much power in the hands of incompetent employers at a time of particular difficulties in scientific employment".

Judy Redfearn

SRC: university cuts are the greatest threat

"The science vote is not being harshly treated" by the Conservative government in the latest round of public expenditure cuts, according to Sir Geoffrey Allen, Chairman of the UK Science Research Council. There is a good case for maintaining a reasonable budget for research, he says, and this is having a strong influence on the government. The science budget is therefore unlikely to suffer the massive percentage cuts proposed for other areas of public expenditure such as education and many of the social services.

The SRC budget is to be cut by only 1½% in real terms in the current financial year and a further 2% in 1980-81. The lack of budget guidelines after 1981, however, is causing problems with medium term planning. For example, the Council's recently announced programmes in post graduate education to improve the performance of graduates in industry (*Nature* 2 August, page 347) are to receive priority over the next few years — but uncertainty in future budgets means that the rate of expansion of the programmes cannot be planned in advance.

Similarly, the SRC cannot plan space and astronomy beyond the end of the current programme in 1983. International collaboration may be one way of cutting costs, says Sir Geoffrey.

The greatest threat to the level of science funding in the UK, however, probably comes from the recently announced cuts in the universities budgets. "These are bound to affect science and engineering", says Sir Geoffrey especially in the short term when running costs will be squeezed. In the long term science and engineering will be cut proportionately less than other faculties and will therefore make a greater part of a smaller whole. "This could be good from our point of view. The government is saying that we've got to be more selective. This means either fewer universities or a smaller overall staff maintaining quality".

Sir Geoffrey hopes that the problem of providing employment for scientists (and coping with any redundancies the universities may have to make) will be helped by the SRC's research fellowship scheme whereby senior scientists can vacate tenured posts by taking up SRC fellowship. "We have the money to do this on a small scale".

Sir Geoffrey's belief in the government's empathy for research was endorsed by the Prime Minister, Mrs Margaret Thatcher, herself last week when she told members of the Chemical Society of her commitment to basic research at a ceremony to award her with the Society's Honorary Fellowship. "One must listen to the voices of scientists and pay attention to what they say", she said. □

Thais put their faith in folk medicine

In first of two articles on scientific issues in Thailand, **David Bousfield** of the University of Sussex describes how religion and medicine often go hand in hand

TRADITIONAL health care systems based on herbal preparations still exist in many developing countries today, and although often despised in the recent past they could provide a focus for the development of local pharmaceutical industries and research. Plants are already important sources of established drugs such as diosgenin (a precursor of many steroids), codeine and atropine. They may also contain compounds with novel actions. The massive screening of plant extracts for anti-cancer drugs carried out under the auspices of the National Cancer Institute, and the research currently being coordinated by the World Health Organisation on physiological bases of folklore contraceptives are good examples of the current interest in this field. In addition, by employing an indigenous health care structure, medical treatment may begin to penetrate rural areas where previously there was no help at all.

South-east Asia provides some useful examples of the possibilities and limitations of integrating this traditional knowledge with the medical needs of a developing society.

In Bangkok alone there are more than fifteen traditional medical schools, some of which can be found on the grounds of Buddhist temples. Wat Poh, the temple of the Reclining Buddha, for example, has long been an important centre for folk medicine in Thailand. In the provinces, such as Chiang Mai in the north, herbal pharmacies are common and are often found beside, or even inside, their modern counterparts.

Certain Buddhist monks, the 'maw phra' or 'doctor bhikkhu', play an important role in treating rural sick. In some cases these schemes can be very ambitious. The Abbot, Phra Chamroon Parn Chan, of the temple Wat Tham Krabok has recently been given the Ramon Magsaysay Award (a south-east Asian equivalent of a Nobel Prize, named after the late Filipino President) for his work with drug addicts. His approach relies heavily on herbal infusions 'to clear poisonous drug residues from the body', thus eliminating 'the physical desire for narcotics'. With government subsidies the Phra hopes to be able to treat up to 4,000 addicts annually.

Doctor Bhikkus self-trained bare-foot 'tambol' doctors are very popular among the lower educated, lower income groups in both rural and urban districts. Several government training schemes are in



Wat Poh temple, Bangkok, which houses a number of traditional medical schools

existence which aim to improve this service in areas such as nutrition and family planning. Use of herbal medicines is taught in many university departments of pharmacy and pharmacology, and in some cases students may even attend additional courses at their local traditional school of medicine.

Research is well under way on the extraction, identification and characterisation of the physiological, pharmacological and toxicological properties of the active principles present in a number of crude drugs. Particular areas of interest include the studies of alkaloids present in local *Rauwolfia* species, some of which could provide a commercial source of drugs such as reserpine. 'Kinchai', Chinese celery (*Anium graveolens*), is also being studied for its potent hypotensive actions and its dramatic ability to reduce temporarily sperm count in man. In Chiang Mai research is being carried out on extracts from a variety of plants including a *Curcuma* (Zingiberaceae), locally known as 'Wan Ngu'. This contains an inhibitor of cobra and viper neurotoxin, and the study of the properties of this extract and its active ingredients has stimulated an exciting amount of neurophysiological and neuropharmacological research. The universities of Mahidol and Chulalongkorn in Bangkok are carrying out even more intensive studies.

The development of useful drugs based on natural product 'diospyrol' serves as a good example. Roundworms, hookworms

and other parasitic infections are a serious health problem throughout south-east Asia. In Bangkok's slums 2% of the children have *Ascaris* infections and 6% hookworm. In rural areas the prevalence of hookworm can exceed 80%, with anaemic complications in half the cases. Effective drugs for treatment do, of course, exist but are often unavailable in the more remote parts of the country. On the other hand, the tree 'Ma-gluea' (*Diospyros mollis*, Griff) which is indigenous to northern and central Thailand has long been recognised for the anthelmintic activity of its berries. The fresh fruit is crushed with a small amount of water, strained, and mixed with coconut water to mask its disagreeable taste.

Clinical trials have shown that the drug is effective against a wide variety of intestinal parasites, and the limited field testing which has been carried out has met with encouraging results. The plant, however, only bears fruit in the rainy season (June to October) and the remedy is only effective if the berries are fresh. A good deal of work, conducted mainly by the Ministry of Public Health in collaboration with Japan, has therefore gone into identifying the active principle, 'diospyrol', a methylated binaphthyl compound, and to discovering methods of preserving the extract. This work was completed in the late 1960s, but since then little has been done to develop herbal medicines to a commercial level.

Research into natural products and herbal medicines is being carried out on a similar scale in at least nine neighbouring countries. These are Thailand's four partners in ASEAN — Indonesia, Malaysia, Singapore and the Philippines — plus Japan, Korea, Hong Kong, Australia and New Zealand. Together they make up the Network for the Chemistry of Natural Products in South East Asia, a Bangkok-based organisation funded mainly by UNESCO and the Japanese government. It is, however, clear that serious problems occur whenever the commercial exploitation of a natural product medicine becomes a possibility.

Ironically this may be the result of the United Nations Industrial Development Organisation (UNIDO) plan for the establishment of drug industries in Third World countries, which involves a backward integration approach to drug manufacture. UNIDO feels that each country should begin with setting up packaging plants, then move on to formulation of imported bulk drugs, and finally manufactures the bulk drugs themselves. But in countries such as Thailand such an approach could stifle the motivation behind much of the current research into herbal medicines and result in many of these new compounds being developed and sold by the multinational pharmaceutical companies. □

Population boom blunts Pakistan's self-sufficiency drive

Despite Pakistan's efforts to become self-sufficient in food grain, it is still falling short of the target every year. The resulting heavy expenditure on the import of wheat, the staple foodstuff, is compounded by considerable congestion at the port of Karachi and in trains carrying the millions of tons of wheat grain upcountry. The whole economy of Pakistan is suffering in consequence. Last year, for example, wheat created a crisis when the country had a bad harvest and imports rose to 2.3 million tons. The problem is not that production strategy is failing for a valid reason — such as targets being unrealistic or the potential being weak — but that the mechanism is inefficient and the will is missing for many imperative measures. These include the package of improved technology for different areas of Pakistan (a target now well within reach) and a streamlining of the distribution of seeds, fertilizers, pesticides and water to the farmer.

At the International Seminar on Wheat held in Islamabad last August, Dr Norman Borlaug, the Nobel laureate, said that at present only one-third of Pakistan's potential wheat production is being exploited. Borlaug, who has been intimately associated with the Green Revolution in Pakistan, blamed the existing official apparatus both for neglecting to apply the available improved technology to wheat production, and for the continued short-sighted reliance upon food aid and concessional purchases (under easy payment contracts from friendly countries, such as the US PL-480).

The pity is that Pakistan also happens to be one of the few countries that staged the Green Revolution early, in the mid-1960s. It had such tempo and potential that Borlaug at that time not only forecast self-sufficiency in food grain within a few years time, but also called Pakistan the 'Mexico of Asia', visualising that it would be exporting wheat and rice to other countries sooner than many imagined. Borlaug's dream was never realised; and sooner than elsewhere, the Green Revolution tapered off.

This is clearly borne out by the wheat production figures since 1965, when the dwarf, high-yield seeds from Mexico became available for commercial sowing.

It can be seen from the accompanying table that wheat production, after nearly doubling from 3.75 million tons in 1965 to 7.10 in 1969, has almost flattened off during the course of the last decade — last year even showing an alarming dip.

Self-sufficiency in wheat could have been attained, even with the very modest increase recorded in the last ten years, but for two inhibiting factors. One is the population explosion, running at a 3% per

annum, a result of the slowly growing standard of living. The *per capita* daily wheat consumption has risen from 244 to 340 g during the last 10 years. Even with a record wheat harvest of almost 10 million tons this year, a short-fall of 0.6 million tons is still possible.

There is however hope that, unless the political situation deteriorates further, Pakistan may now be able to turn the corner. The new factor is a drastic reorganisation of the Pakistan Agricultural Research Council (PARC), which has turned it into a fully autonomous organisation with wide powers of planning, coordination, financing and formulating national strategy for agricultural research. The PARC now has a full-time chairman, with the status of secretary to the government. It is also intended that the should be a scientist of high repute and not a politician. The present chairman, Dr Amir Muhammad, has gained the necessary experience and expertise to organise research on a national level through his previous positions as Vice-chancellor of an agricultural university and Director of the Atomic Energy Agricultural Research Institute.

The PARC is now equipped to provide leadership to other research organisations in the field. This is particularly significant in Pakistan, where agricultural research has largely been fragmented at the provincial level and has lacked direction. This was all too manifest last year in the wheat production debacle. Farmers had sown considerable amounts of wheat varieties that were susceptible to disease, even though disease-resistant types could have been easily identified and the farmer suitably advised — if the research organisations and their provincial extension services had been alive to the situation.

This year's 20% increase in wheat output is partly due to the PARC reorganisation, and partly to the federal government itself. After recovering from the panic of having to import 2.3 million tons of wheat, and paying the heavy price, the government is now taking an active interest.



The PARC has started taking such vital steps as zoning the country for some of the major crops in terms of agro-ecological variations, sending its research teams in different zones to conduct investigations and research on the spot, and providing analytical service facilities not available at the provincial institutes. But its most significant achievement appears to be the establishment of properly constituted National Agricultural Research Centre near Islamabad. A staff of 25 scientists, eight of them postdoctoral, are already working at the centre, with another 40 in the process of recruitment. The emphasis is on high calibre to strengthen the team.

The centre has already mounted a large number of experiments on its own fields (which cover 1,500 acres) involving topics such as plant genetics, agronomy, and plant pathology. It is also investigating the 'package technology' of producing different grains for different areas. Each package will contain information on varieties, cultural practices, fertilizers, weed control, harvesting, and storage.

Genetic resources conservation is another vital responsibility of the centre. It has already collected thousands of exotic, as well as local, varieties of wheat, rice, triticale, maize and other grains. They will be preserved for up to 100 years in the main depository, in vacuum at 0°C. To mount an effective genetic variety programme, the breeding stock should have great genetic diversity; breeders all over the world have started to feel the pinch of starvation as new sources of genetic diversity are getting scarce, squeezed out by the rapidly spreading improved crop plant varieties.

Azim Kidwai

Table 1 Pakistan wheat production since 1965

Year	Wheat output (millions of tons)	Imports (millions of tons)	Population (millions; approximate)
1965	3.75	Not known	5.5
1968	6.50	0.015	
1969	7.10	0.123	
1971	6.78	0.439	6.5
1974	7.50	1.179	
1976	8.55	2.300	
1977	9.00	1.087	
1978	8.15	2.300	7.5
1979	9.99	Not yet known	

news and views

Probing the insulin receptor

from P. H. Sönksen

THE German Wool Research Institute houses some of the foremost peptide chemists in the world who sharpen their teeth on insulin as a simple chemical model for the more complicated molecules that will be keeping us warm in the months to come. At the Institute two young students, Peter Thamm and Achim Schüttler have respectively synthesised a family of specifically photolabelled insulin analogues and made a variety of specific covalently-linked insulin dimers which together have led directly to major developments in our knowledge of the insulin receptor. The insulin receptor was perhaps the most popular subject at the International Insulin Conference* held at the Institute recently.

Remarkably concordant results on its composition were provided by three independent groups. M.H. Wisher and colleagues (St Thomas's Hospital Medical School, London) have specifically covalently labelled a membrane protein of approximately 300,000 molecular weight which can be resolved into a subunit of apparent molecular weight 130,000. This polypeptide runs atypically on polyacrylamide gels and may be a glycoprotein of true molecular weight nearer 90,000. Rather surprisingly this same membrane component was readily and specifically labelled with probes carrying the photoreactive group in the A₁, B₁ or B₂₉ positions.

These observations were exactly in keeping with those of C. Yip (University of Toronto) working independently with similar but less specifically photolabelled probes. Yip reported two specifically labelled components (130,000 and 90,000 molecular weight) in membrane extracts from insulin-responsive cells but only the 130,000 molecular weight components in extracts of cells containing insulin binding sites but no insulin-responsiveness (such as brain) and went on to suggest that the 90,000 component may be an effector molecule. M.P. Czech (Brown University, Rhode Island) using a bifunctional cross-

linking reagent was able to produce almost identical results by covalently linking radiolabelled insulin to the membrane receptor after the two had been allowed to react *in vitro*. He, like Wisher, had also isolated a 300,000 molecular weight component that could be dissociated into 125,000 molecular weight subunits after reduction of disulphide bonds. Early results from C. Diaconescu and D. Saunders (DWI) and A.R. Rees (University of Oxford) suggest that covalent linkage of insulin to its receptor in adipocytes may lead to prolonged activation of insulin-dependent processes.

Other remarkable observations on function of the receptor come from the synthetic dimers (Schüttler). They show considerable discrepancy between receptor-binding activity (100%) and biological activity (considerably impaired, 0–60% depending on assay). P. De Meyts (Institute of Cellular and Molecular Pathology, Brussels) and colleagues

concluded, on the basis of their studies with these dimers, that each monomeric half of the dimers might bind to a component of the insulin receptor and in some way prevent activation (possibly by inhibiting subsequent conformational change). On the other hand K. Willey and colleagues (St Thomas's Hospital Medical School, London) felt that their own results were compatible with the simpler concept of dimer reaction through one component only.

Other examples of the important contribution made by the synthetic chemists include some very elegant experiments reported by A.S. Rosenthal (Merck Sharp and Dohme Research Laboratories, New Jersey).

In the presence of synthetic peptide fragments of insulin, ³H-thymidine incorporation into lymphocyte DNA as a measure of T cell response, was used to delineate the specificity of the phenomenon. In one strain of guinea pigs

More on anthropoid origins

from Peter Andrews

THE origin of the higher primates is a contentious issue at present. Traditional interpretations place the tarsier closest to the anthropoid primates but an alternative to this has been advocated for some years by P.D. Gingerich of the University of Michigan. Gingerich has demonstrated a nearly continuous series of fossil primates from the early Eocene to the Oligocene all belonging to the family Adapidae (for a review see *J. hum. Evol.* 6, 483; 1977), and he has shown that these become adaptively similar with time to early anthropoids.

In this issue of *Nature* (page 65) a group of Burmese and American anthropologists have described a new specimen of fossil primate from the late Eocene of Burma. This is named *Pondaungia cotteri*, and it possesses a number of specialisations that suggest it had reached the anthropoid grade, specialisations such as the great depth of the lower jaw, and the cusp and wear patterns of the teeth. These specialisations are overlaid on a pre-anthropoid pattern that seems to be most similar to the generalised adapids of Europe and Asia that Gingerich advocates as the immediate precursors of

the Anthropoidea, and if these conclusions are substantiated by further discoveries it might well be possible to locate the origin of the anthropoids in the late Eocene of Asia. Work is continuing in Burma, and it is to be hoped that Ba Maw and Russell Ciochon will find additional specimens.

By the middle of the succeeding period, the Oligocene, anthropoid primates are represented by a number of species in the Egyptian Fayum deposits, which are currently being excavated by Elwyn Simons of Duke University, and by the end of the Oligocene they are also known in tropical Africa. They diversified during the succeeding periods up to the present, giving rise to all the living species of monkeys and apes and man. The adapids on the other hand diminished in numbers after the end of the Eocene, with the latest known population occurring in 10 million year old deposits in India and Pakistan where they apparently existed as a relic population (described earlier this year by Gingerich in *Nature* 279, 415; 1979).

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*The 2nd International Insulin Symposium was held at the Deutsches Wollforschungsinstitut, Aachen on 4–7 September, 1979, and organised by Professor H. Zahn and Dr Dietrich Brandenburg.

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T cell response could be localised to a few amino acids around the tenth residue of the B chain. On the other hand the specificity of antibodies produced by these animals did not reflect the specific determinants

unique to the insulin used for immunisation. This distinction between T cell response determinants and specificity at the serum antibody or B cell level was also shown in mice. □

Nuclear quadrupole resonance at Toulouse

from J.A.S. Smith

P. GRIVIT (Université de Paris), in whose laboratory the first nuclear quadrupole resonance experiments in France were conducted, opened the conference*. He reviewed in masterly fashion the many current applications at Orsay of nuclear quadrupole interactions to solid state physics, concluding with the description of a sensitive pulsed spectrometer designed by L. Guibé and his coworkers (Paris, Orsay), a feature of which was its highly automated operation. This theme was repeated in several other papers, notably from E.A.C. Lucken's group (University of Geneva) who described a pulsed FT spectrometer for the study of single crystals, from which the complete electric field gradient tensor may be derived under computer control. Other workers described frequency and phase-locked spectrometers for the determination of true line shapes (P.T. Narasimhan, Indian Institute of Technology, Kanpur) and a special pulsed coil system for detecting ^{14}N signals from samples external to the spectrometer (T. Hirschfeld & S.M. Klainer, University of California, Berkeley) with potential applications to geochemistry. A recent development, the derivation of nuclear quadrupole interactions in free radicals by ENDOR experiments, was described by A. Colligiani (CNR Quantum Chemistry Laboratory, Pisa), who reported the first measurement of the full ^2H quadrupole coupling tensor in the free radical HOOC.CD.COO^- .

The applications of nuclear quadrupole resonance to transition metal chemistry were emphasised. T.B Brill (University of Delaware,) described the applications of ^{55}Mn quadrupole resonance to the study of the electronic structure of tricarbonylcyclohexadienylmanganese(I) and its substituted analogues, and other speakers discussed ^{63}Cu resonance in diamagnetic copper(II) complexes (T. Okuda & A. Weiss, Technical High School, Darmstadt), ^{35}Cl quadrupole resonance in mercury pentachlorocyclopentadienes (G. Wulfsberg and coworkers, St John's University, Collegeville, Minnesota), in some hexachloroplatinates (IV) hexahydrates (A. Sasane, Shinshu University, Japan), and

Zeeman studies of ^{63}Cu resonance in single crystals of Cu(I) triphenylphosphine dimers (H. Negita *et al.* Hiroshima University, Japan). Although it has been known for some time that quadrupole resonance of ligand nuclei can be readily studied in paramagnetic complexes, for example ^{14}N quadrupole resonance in dichloro (dimethylnitrosoamine) copper (II) (D. Nakamura, Nagoya University, Japan), T.J. Bastow and H.J. Whitfield (CSIRO, Clayton, Australia) showed in their paper that strong ^{63}Cu signals can be detected in several paramagnetic Cu(II) salts such as CuCl_2 and CuBr_2 ; the signals are seen because of strong exchange narrowing, with widths of about 35 kHz, little different from that of ^{63}Cu resonance in diamagnetic Cu_2O . In a review of recent studies of inorganic coordination compounds, T.L. Brown (University of Illinois) discussed the wealth of information now forthcoming from solid complexes by a combination of conventional cw and pulsed measurements with double resonance techniques.

The remarkable increase in sensitivity afforded by double resonance experiments was emphasised by many speakers. For ^2H and ^{14}N , the technique is now well established; J.L. Ragle (University of Massachusetts, Amherst) demonstrated the usefulness of ^2H quadrupole resonance in a study of pyridine and its salts, and ^{14}N double resonance was applied by M. Garcia (Queen Elizabeth College, London) to many imidazole derivatives and by A. Loetz and J. Voigtländer (University of Munich) to a series of amidinium salts. Three papers were devoted to another recent development of double resonance spectroscopy, that of the detection of ^{17}O quadrupole resonance signals in natural abundance; J.A.S. Smith (Queen Elizabeth College, London) and S.G.P. Brosnan and D.T. Edmonds (University of Oxford) discussed the novel features of such methods applied to compounds containing -O-H groups, the most important of which is the appearance of dipolar structure, and O. Lumpkin (University of California, San Diego) described its applications to compounds containing O-O bonds, such as $\text{IrO}_2\text{Cl}(\text{CO})(\text{PO}_3)_2$ a reversible oxygen carrier. In a later paper, P.J. Bray (Brown University, Rhode Island) demonstrated

very clearly the significance of ^{14}N quadrupole coupling constants as a measure of biological activity, for example in the bacteriostatic activity of the sulphanilamides and the tumour-inhibiting properties of several nitrogen mustards.

Studies of phase transitions and molecular motion in solids were common themes of a number of papers. J.R. Brookeman (University of Florida) gave a timely review of the importance of measurements of the resonance frequency and relaxation times close to phase transitions and several other papers discussed the temperature dependence of the same quantities for example, in hydrazine derivatives (Y. Abe, University of Tsukuba, Japan), in some bromocyclo-triphosphazatrienes (A.L. Porte, University of Glasgow) and in ammonium salts such as $\alpha\text{-NH}_4\text{HgCl}_3$ and NH_4ReO_4 (H. Chihara, Osaka University, Japan) in which the anomalous temperature coefficients were interpreted by taking into account the finite jump-time of the NH_4^+ ion between its equilibrium configurations. There was increasing interest in the value of variable pressure studies in studying solid-state processes; R.J.C. Brown (Queen's University, Kingston, Canada) stressed the generality of the relationship between the temperature and pressure coefficients of the quadrupole resonance frequency and J. Stankowski (Poznań, Poland) studied ^{75}As resonance in $\text{K D}_2\text{AsO}_4$ up to 3kbar as a test of current theories of proton-ordering in this crystal. I.G. Shaposhnikov (Perm, USSR) discussed a new theoretical attempt to analyse the 'Bayer' problem, the temperature variation of the quadrupole spin-lattice relaxation time, T_{1Q} , due to random thermal oscillations, by the methods of quantum kinetics; his theory shows that significant departures of T_{1Q} from the predictions of the Bayer equations can be expected at temperatures above $T_{m/5}$, T_m being the temperature at which orientational order disappears. Experimental aspects of relaxation time measurements were referred to by A.H. Brunetti (IMAF, Cordoba, Argentina) and there was some discussion of the usefulness of T_2^* , the spin-phase memory time, by S. Hacopian (University of Sydney) and M.A. Whitehead and coworker (McGill University).

Nuclear quadrupole resonance spectroscopists have long been worried about high-order nuclear electrical multipole contributions to their measured frequencies; S.L. Segel (Queen's University, Kingston) showed by careful frequency measurements on a number of nuclei such as $^{185,187}\text{Re}$ in NH_4ReO_4 , ^{209}Bi in BiCl_3 and ^{93}Nb in NbCl_5 that there were no measurable hexadecapole contributions. The question as to whether this is generally true still remains. □

*The fifth international symposium on Nuclear Quadrupole Resonance Spectroscopy was held in Toulouse, France on 10-14 September, 1979. The co-chairmen were L. Guibé (Université de Paris) and G. Jugie (CNR, Toulouse).

J.A.S. Smith is Professor in the Department of Chemistry, Queen Elizabeth College, London.

Urban ecology

from Peter D. Moore

THERE are areas of ecology where the development of theoretical models has outpaced the accumulation of the field data necessary to test them. Often the observations which exist are not in the appropriate form, and models stand unchallenged until the required information is gathered. One conceptual model which has received much attention from various quarters in recent years is the idea of island biogeography and its applicability to fragmented habitats of all kinds. The original statements concerning the immigration, extinction and stabilisation of island biotas were tested experimentally by Simberloff and Wilson (*Ecology* 50, 267; 1969), but since then the model has been used extensively in ecology, for example in the determination of the size and spacing of nature reserves (see May *Nature* 254, 177; 1975). Although, theoretically the model can be used in any fragmented habitat where intervening areas are unsuitable for settlement, experimental or even detailed observation concerning its wider applicability are generally few. Some such applications are mountain tops (Brown *Am. Nat.* 105, 467; 1971), fresh water habitats (Sepkoski & Rex *Syst Zool.* 23, 165; 1974), oak trees and leaf-mining insects (Opler *Am. Scient.* 62, 67; 1974) and cushion plants (Tepedino & Stanton *Oecologia* 25, 243; 1976).

One type of habitat in which island biogeographic theory could be put to the test is the mosaic of wasteland produced by demolition in city centres. Here are sites which are available for colonisation by weeds from surrounding sources, invading across unfavourable intervening terrain. Often the age of such sites can be precisely established and it could be argued that a level of equilibrium between immigration and extinction should be established relatively rapidly. If this were so, then theoretical considerations would lead one to postulate that the number of species at a site should be a function of the site area and should be inversely related to the distance from the origins of immigrant flora.

Crowe (*J. Biogeog.* 6, 169; 1979) has examined the potential of such sites in Chicago. He surveyed 26 vacant urban lots in the city, establishing a plant species list for each site in addition to the determination of lot area, age, distance to the nearest older lot and lot density in the area, among other parameters. A disadvantage of a single survey rather than a long term study of each site is that one cannot be sure that species equilibration has occurred; nothing is known of the actual pattern of immigration and extinction. Crowe demonstrates that the vast majority

of species found in the younger lots have survived in older sites also, so the system has not reached a successional stage where competitive processes are leading to extinction. This is further confirmed by the fact that the species number in a lot is closely related to site age in the younger lots, but for older ones (greater than four years) there is little increase with time. This indicates, but does not fully prove, species equilibration.

The number of species in these older equilibrated lots is positively correlated with lot area and to the density of other lots in the vicinity (number of lots within 1 km). It is negatively related to the distance to the nearest lot. These two latter features are indices of isolation from potential sources of immigrant plants. Thus the conclusions reached are in keeping with the predictions of island biogeographic theory.

One further variable which requires consideration is the diversity of micro-

habitats available within any lot. Crowe has not attempted any such analysis, but this aspect of the island biogeographic model has been looked into by Simberloff (*Ecology* 57, 629; 1976), who concluded that although habitat diversity is an additional complicating factor, if one can eliminate this variable experimentally there remains a residual relationship between number and island area.

Observational data, however, may not permit such a fine resolution of the influence of island area and habitat diversity, for the two are frequently themselves positively related. Thus, Juvik and Armstrong (*J. Biogeog.* 6, 205; 1979) when examining the biogeography of the endemic honeycreepers (Drepanididae) of the Hawaiian archipelago, found a positive correlation between island area and species richness. But since larger islands are also more diverse in their habitats, generally having the higher mountains and more

Nutrient retention in tropical rainforests

from Robert M. May

IN the hour you might devote to reading this issue of *Nature*, more than 3,000 acres of tropical rainforest will be destroyed. According to the Food and Agriculture Organization of the United Nations, there were about 16 million km² of rainforest around the turn of the century, which by 1975 was reduced to 9.4 million km², representing a 40% reduction; a conservative estimate of the current loss rate is 27 million acres per annum, or 50 acres per minute. This mounting tragedy of the world's rainforests is an oft-told tale (two recent and powerful articles are by Lovejoy and Colinvaux in *Nature Conservancy News* 29(4), 1979; see also *Nature* 257, 737; 1975), and I intend here to dwell only on one narrow aspect of it.

Many lowland rainforests grow on very poor soils. These acid soils have low capacity for ion absorption, making them particularly susceptible to the leaching of all plant nutrients. Rainforest trees have responded to this by storing most of the nutrients of the forest in the trees themselves, and by evolving symbiotic associations with mycorrhizal and other fungi that recover and recycle those nutrients that make their way into the soil when a tree dies or when its parts are eaten and excreted by animals. Colinvaux extols these fungi, "Whose pervading presence contributes to that characteristic greenhouse smell of the forest. The smell of the fungi is the smell of life — the part of the system that circulates nutrients. When a rainforest is cleared, the smell is gone, and so are the nutrients that fungi and rootlets once conserved to make productive life possible."

Despite their importance, these ideas

about direct cycling of nutrients in rainforests have only recently received direct experimental verification. In a most elegant study, Stark and Jordan (*Ecology* 59, 434; 1978) added radioactive ⁴⁵Ca and ³²P to the surface of experimental plots in the Amazonian rainforest, near San Carlos on the Rio Negro in Venezuela. These radioactively labelled nutrients were added both in leaves and twigs placed on the forest floor, and as a direct chemical spray (simulating 'decomposed' litter) on the plots. The fraction of this radioactive material that leached through the root mat and humus layer was monitored for 6 months. In 9 of the 10 study sites, less than 0.1% — one part in a thousand — of the labelled nutrient leached past the root mat, and all leaching ceased after 1 to 2 months; in one of the 10 plots, slightly less than 1% of the radioactive material leached through. In addition, Stark and Jordan analysed root mat samples, showing directly that the radioisotopes were indeed taken up and translocated by living roots.

Noting that the history of the Amazon Basin and other tropical rainforests is filled with failure after failure to establish large-scale agriculture, tree plantations or pasture, Stark and Jordan are concerned to emphasise the message substantiated by their experiments: "The exploiters reason that an area that can support a luxurious forest should be able to support permanent agriculture. This study has demonstrated that this is not the case, because of the evolved and unique root mat which enables the undisturbed forest to survive." □

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'ecozone patches', it proved impossible to extricate the pure area influence from that of habitat heterogeneity. Evidently this aspect of the island biogeographic model needs more attention in order to understand the area/richness relationship whether at the urban lot or the Hawaiian island level of organisation. □

Mapping the nuclear potential

from P.E. Hodgson

THE special characteristics of nuclear heavy-ion interactions enable the nuclear surface to be probed in detail, and this has recently been used by Nees and Guidry of Oak Ridge and Donangelo and Rasmussen of Berkeley (*Phys. Lett.* **85B**, 201; 1979) to map the deformed potential of the nucleus ^{160}Gd .

To do this, they analyse the inelastic scattering of ^{40}Ar by ^{160}Gd at energies comparable with that of the Coulomb barrier. This interaction excites several states in the ground-state band, and there is strong interference between the Coulomb and the nuclear forces. At these energies, the wavelengths of the ions are small, so that semi-classical theory can be used to understand the interaction. This shows that since both Coulomb and nuclear forces contribute to the excitation, both the charge and the matter distributions influence the interaction, and are thus both probed by it. At energies below the Coulomb barrier few non-elastic processes take place, and so the effects of the real and imaginary parts of the potential can easily be separated.

From the uncertainty principle we know that localisation in angle requires a large uncertainty in the momentum. If we consider the experimental situation as shown in Fig. 1, when scattering is

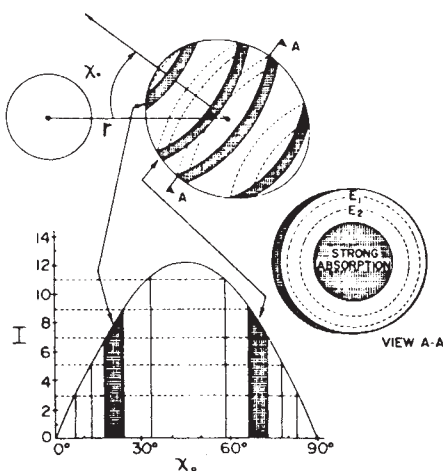


Fig. 1 Schematic illustration of the radial and angular localisation in heavy ion reactions. The more classical the system the more the surface interaction is localised in angle and in radial distance.

restricted to backward angles, we find that the transfer of multiple rotational quanta implies a localisation in the orientation angle of the deformed rotor during the interaction, and hence a localisation in the part of the nuclear surface probed by the projectile. Classical phase space arguments show that each classical angular momentum I_i is associated with a range of initial orientations obtained by projecting two units of I_i on the angular axis, as shown in the figure. This spread in spin corresponds to a definite range of initial orientation angles, so that a particular spin state probes specific regions of the deformed nuclear surface.

Similar arguments show that a large radial angular momentum implies localisation in the radial coordinate. Thus the initial width of the radial wave packet for ^{40}Ar on a heavy nucleus at the energies of these measurements is about 0.1 fm. The projectile spends most time in the region of the classical turning point, and so the interaction probes this region of the nuclear surface. As the incident energy increases the turning point moves inwards and with it the region being probed, until the particle is absorbed by the strong interaction in the nuclear interior.

These qualitative arguments are of course confirmed by numerical calculations that are fitted to the cross-sections for the inelastic scattering with the excitation of the low-lying states of the ground-state rotational band. A complex deformed Saxon-Woods potential was used, with fixed potential depths, and the radius and diffuseness parameters refer only to the localised region probed by the particular interaction.

From the results of these calculations the real part of the potential is constructed piece by piece, assuming axial and reflection symmetry. It is found that for each quadrant the classically allowed states (4^+ and 6^+) sample two regions while the classically forbidden ones (8^+ and 10^+) sample one broad region. The first three diagrams in the figure show the partial contributions of each state, and the final one the sum. It is notable that the contours overlap and join very smoothly, even though each comes from an independent analysis of a different part of the potential.

These potential contours can easily be parametrised in terms of an expansion in spherical harmonics to give a precise description in the usual form. They are closely related to the contours of the nuclear density distribution itself through a simple folding calculation with the nuclear interaction. Conversely, they may be used to check the accuracy of any particular form of the nuclear surface. This method of analysis of heavy ion interaction thus gives a very direct and clear description of what it means for a nucleus to be deformed, and should have many useful applications to studies of nuclear structure. □

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Bird colours

from John R. Krebs

MOST biologists, if asked to hazard a guess about the possible significance of the brilliant plumage adornments of many bird species, would probably say that bright colours evolved in the context of ritualised display between rival males or as attractants to lure females. They would point to the fact that male birds are usually brighter than females, and exotic male plumage is especially highly developed in polygamous birds. Males generally compete for females, and this competition is more intense in species where one male can acquire a larger number of mates, so sexually selected colours are expected to be found in males, and especially in polygamous males. This was the line of argument put forward by Darwin, and although a number of alternatives have been proposed from time to time, the first serious attempt to examine Darwin's idea by means of a large scale comparative survey has recently been published by Baker and Parker (*Phil. Trans. R. Soc.*, **B287**, 63-120; 1979). As a result of their analysis, Baker and Parker reject the sexual selection hypothesis and replace it with what at first sight may seem a surprising idea: that bright colours have evolved in response to selection pressures exerted by predators.

More than 30 years ago, Cott published an extensive study showing that bright coloured birds are often distasteful (*Proc. Zool. Soc., Lond.* **116**, 371-524; 1946). His work started with an observation made while preparing museum skins in the Middle East. Cott noticed that hornets readily ate the flesh of cryptic birds such as the palm dove (*Streptopelia senegalensis*) and ignored the carcass of a conspicuous pied kingfisher (*Ceryle rudis*). This led Cott to a study of the hornet's preferences for the flesh of 38 Middle Eastern birds ranging from the very cryptic wryneck (*Jynx torquata*) to the conspicuous white crowned black wheatear (*Oenanthe leucopyga*). These, and later tests with cats, and a survey of human gastronomic preferences, confirmed Cott's thesis that bright colours are often associated with unpalatability. The phenomenon of warning (aposematic) colouration is well known in insects, and Cott extended the idea to birds. Baker and Parker point out that distastefulness is not the only basis for aposematic colouration: if a prey item is hard to catch, bright colours may evolve as a signal of unprofitability. They suggest that this might be especially applicable to birds, which once they have seen a predator can manoeuvre fast enough to be almost certain of escape. Thus the bright colours of many birds might signal not only "I am distasteful" but also (or alternatively) "I am hard to catch" or "I have seen you". As with the conventional theory of aposematic colouration, Baker and Parker's thesis assumes that bright, as opposed to

FIVE years ago few biologists would have predicted that the structural organisation of eukaryotic genes would be known in such great detail. However, although we now know the primary structure of several genes, the mechanisms by which their transcription is controlled remain unclear. Speakers at the fifteenth Harden conference* set out to bridge this gap between our knowledge of structure and its relation to function.

One system which we are on the way to understanding is the transcription and processing of yeast tRNA genes. The primary transcript of these genes is trimmed at both 5' and 3' ends and a short intervening sequence is removed to produce the mature tRNA. J. Abelson (University of California, San Diego) reported work in progress to purify the enzymes responsible for splicing out the intervening sequence, using as a substrate precursors purified from a temperature-sensitive mutant strain deficient in processing. A specific endonuclease and ligase have been separated, which are unusual in that they respectively create and rejoin a 3' terminal phosphate and a 5' hydroxy group. E. de Robertis (MRC Molecular Biology Laboratory, Cambridge) reminded us that tRNA precursors microinjected into *Xenopus* oocytes can be completely processed (*Nature* 278, 137: 1979). That toad enzymes can correctly splice out the yeast tRNA intervening sequence is surprising since it has diverged markedly in sequence from that of *Xenopus*. B. Hall (University of Washington, Seattle) has taken advantage of the ability of *Xenopus* enzymes to process yeast tRNA correctly to probe the relationship between structure and function, by studying the transcription and processing of cloned mutant tRNAs in a *Xenopus* cell-free system. Twenty mutants selected by loss of nonsense suppressor activity, have been cloned and sequenced. Interestingly, all were found to lie within the 5' and 3' limits of the mature tRNA. One mutant, located in the intervening sequence, was not correctly transcribed and gave a primary transcript

Structure and function of eukaryotic genes

by Jonathan Wolfe

only 48 nucleotides long.

This type of 'surrogate' genetics can clearly provide information about the influence of primary structure on transcription and processing; however results obtained in heterologous systems should be interpreted cautiously. J. Beggs (Plant Breeding Institute, Cambridge) showed us that not all yeast and vertebrate systems are mutually compatible. She transformed yeast with a plasmid carrying the rabbit globin genes and flanking sequences. Globin transcripts were made: however the yeast did not splice out the first intervening sequence and transcription terminated within the second. This point was also made by C. Weissmann, in the Harden Lecture, who stressed the value of studying transcription and translation in a homologous system.

The yeast tRNA genes were not the sole topic of the meeting. C. Henschel (University of Zurich) reported results from M. Birnstiel's group who have now almost completely sequenced the sea urchin histone genes. They have found a Hogness box, the putative eukaryotic promoter, a short distance upstream of the H2A gene and have constructed a number of plasmids carrying small deletion mutants of this box or of the surrounding sequences. Sea urchin DNA supercoils, removed from the plasmid vector, were then tested (in *Xenopus* oocytes) for their ability to be transcribed and translated. Deletion of sequences between the Hogness box and the AUG codon of the H2A mRNA was found to have little effect on the amount of transcription. However, removal of the Hogness box greatly reduced transcription and led to variation in the point of initiation. What, at first sight, were contradictory results were

Bendig and W. Folk of the University related by R. Kamen (Imperial Cancer Research Fund, London) reporting work with a deletion mutant isolated by M. of Michigan. He told us that deletion of an apparent Hogness box upstream of the polyoma virus early region genes actually led to an increase in the rate of transcription initiated at a new site upstream of the deletion. However, the deletion extended into the 5' end of the message and may have decreased the rate at which it was translated. One possible explanation of this result was therefore that the early region is under feedback control exercised by one of the protein products and the reduction in this inhibition may more than cancel out any possible decrease in transcription as a result of deletion of the promoter.

Surrogate genetics is clearly a powerful tool to investigate structure-function relationships in straightforward systems but it is of limited value when probing more complex situations. However, we were provided with one example of the rapid progress which molecular biologists can make when working with a complex system already well-characterised genetically. K. Nasmyth (University of Washington, Seattle) reported work in which he has confirmed the geneticists' 'cassette' model of yeast mating type switching. This model explains the 'switch' in mating type from α to a or vice versa which can occur in budding yeasts, by proposing that silent copies of the two mating type genes exist elsewhere in the genome (at loci HML α and HMRA) and that they can reprogramme the locus at which the active mating type is expressed (MAT). Nasmyth has now shown common sequences at the MAT locus and at the loci HML α and HMRA. Clones were selected for their ability to complement MAT α yeast, which are unable to respond to mating type a hormones and it was then straightforward to clone all three loci. Heteroduplex mapping showed that although the clones had sequences in common they contained a or α -specific sequences and also sequences specific to each locus. □

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*Held at Wye College, Ashford, Kent UK on 16-21 September, 1979, and organised by Professor Peter Walker on behalf of the Biochemical Society.

dull, colours are needed to signal a warning, (although there is no evidence that predators learn faster to avoid bright colours). There is also a possible problem in explaining how the genes for warning colour spread in the first place, since each predator has to attack, and perhaps kill, a few individuals to learn its lesson.

Baker and Parker also suggest that some bird colours are used as 'flash colouration'. These are colours of otherwise cryptic animals which are exposed suddenly when a predator attacks. The predator is tem-

porarily startled or confused, allowing its potential victim to escape. The bright red eye and black and white head of the night heron (*Nycticorax nycticorax*) might be such an example.

The evidence adduced by Baker and Parker for their hypothesis consists of a comparative survey of 516 species of western Palaearctic birds. For each species they coded conspicuousness on a scale of 0-5 for different regions of the body — crown, head, neck, back, belly and so on. They then carried out a stepwise multiple

regression to look for correlations between conspicuousness of each body region and various ecological and life history variables such as diet, gregariousness, mating system, migration distance, and nesting behaviour. The analysis was done separately for adults, juveniles, males, females, summer and winter plumage. The authors admit that there are many inadequacies in their analysis, not least of which is deciding how conspicuous a bird is in its natural habitat.

I can mention only a few of the many

results produced by the analysis. As expected, males emerged as more conspicuous than females, and the dimorphism was more marked in polygamous than monogamous birds. These results seem to fit nicely with the classical sexual selection hypothesis, but according to Baker and Parker they do not. For example, within the polygamous species, only those in which the males display on open ground with a good view of approaching predators are bright coloured. This might be seen as supporting the idea that the bright plumage signals to the predator "I have seen you". Crypsis of females is generally related to their greater role in parental duties, and where males perform parental care they also tend to be cryptic, at least in the parts of the body most visible to predators. Because an incubating bird is more likely to be caught by surprise, it relies on crypsis rather than aposematic colours to escape predation.

A similar argument is used by Baker and Parker to account for the difference between diurnal and nocturnal birds. Diurnal birds are more conspicuous because they are usually alert to the approach of predators and are therefore unprofitable prey. Nocturnal birds rely on immobility and crypsis to avoid being eaten while they are asleep during the day, but they sometimes have flash colours which are revealed when the resting bird is disturbed. In this respect there is a parallel between birds and Lepidoptera, where diurnal forms are bright coloured and nocturnal species cryptic.

As with any comparative study of this sort, a major problem in the interpretation of Baker and Parker's results is to distinguish between cause and effect. Consider, for example, two polygamous species, the ruff (*Philomachus pugnax*) and wren (*Troglodytes troglodytes*). The former is strongly dimorphic and lives in open habitats where the conspicuous males display on communal ground. The wren is monomorphic and the cryptic males defend large territories in dense vegetation. According to Baker and Parker's thesis,

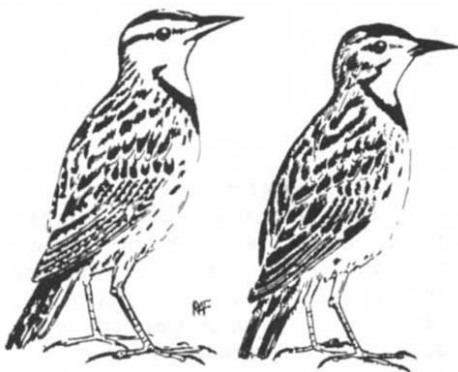


Fig. 1 Remarkable plumage convergence. The eastern meadowlark (left) is an American Icterid, while the yellow throated longclaw (right) is an African Motacillid, entirely unrelated.

male ruffs, as a result of living communally in open areas, are vigilant and therefore unprofitable to predators. Their bright plumage advertises their unprofitability. Male wrens in dense vegetation are easily surprised by a predator, and hence rely on furtive habits and crypsis to survive. But the interpretation can easily be turned the other way round. Suppose bright colours are always favoured by sexual selection, but that predation acts as a constraint to prevent sexually selected colours developing in some species. In the vigilant ruff the balance of these opposing selection pressures is in favour of sexual selection, while in the more vulnerable wren, predator selection has the overriding influence.

Baker and Parker restrict their analyses to overall differences between species in conspicuousness of particular body

regions. Equally intriguing are remarkable convergences in the fine detail of plumage between unrelated species occupying similar ecological niches in different parts of the world. The African yellow throated longclaw (*Macronyx croceus*) and the North American meadowlark (*Sturnella magna*) constitute perhaps the most remarkable pair (Fig. 1), but there are many others. It is tempting to conclude that ecological selection pressures must influence the fine detail of plumage as well as overall conspicuousness, and a comparative survey like that of Baker and Parker could be a way of testing this conclusion. □

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100 years ago

Several German newspapers strongly deprecate the undignified tone which seems to have reigned amongst the participants in the recent celebration at Pompeii. It appears that the majority of the visitors treated the whole matter as an excellent joke, and behaved very much as they would have done at a fair or at some other Neapolitan fête. Many of the archaeologists who had come long distances to be present at the celebration, left the dead city in disgust at the behaviour of the multitude, long before the proceedings were half over. One of the articles referred to expresses the hope that in the year 1979 the Neapolitans will have sufficiently improved in manners as well as gained in seriousness, to render a repetition of the disgraceful scene impossible.

The London correspondent of the *Scotsman*, who is usually particularly well informed, states the Treasury has definitely decided not to ask Parliament for the cost of fitting up the new Natural History Museum at South Kensington and for the removal of the Natural History Department of the British Museum thereto till 1881. We are glad to be assured, however, that this statement should only be received as a rumour, and is not yet sufficiently authenticated. Let us trust that it will not be authenticated at all.

For the first time perhaps in the history of electric lighting two rival magneto-electric machines are illuminating the same hall. The Lontin and Siemens generators and lights are exhibited at about 120 yards from each other in the large hall of the Palais de l'Industrie, at Paris. Each electrical machine is worked by steam, and consumes a certain amount of horsepower. The competition is too recent to offer yet any definite opinion on the respective merits of the apparatus confronting each other.

Telegrams from Murcia state that the city and celebrated *Muerta* were inundated by an

immense waterspout which was formed in the sea at a distance from the coast on the night of October 14-15. Salt water was discovered at a distance of 45 kilometres from the sea. Another great atmospherical commotion was experienced three days afterwards. A night snowstorm enveloped the whole of Austria and Switzerland, and in Vienna the thickness of the snowfall was several inches. It is the first time since 1852 that snow has fallen so early in Vienna, but not the earliest time on record, as in 1760 it fell on October 12.

Mr. C. P. Edison, nephew of the great American inventor, has just died at Paris at the early age of twenty-four. He was his uncle's principal assistant in the production of the loud-speaking telephone, and was sent over to London by him to exhibit that instrument before the Prince of Wales, the Royal Society, &c. He had of late been engaged in applying his uncle's system of quadruplex telegraphy between Paris and Brussels.

The sanitary congress

This Congress continued its meetings at Croydon during last week, when several interesting addresses were given. Mr Douglas Galton, in his address last Thursday, spoke of that large class of conditions which are the direct result of the circumstances to which man is exposed in consequence of living in communities. All living beings are in a continual condition of change, which results in their throwing off from the body matters which poison earth, air, and water, unless space, time, and opportunity are afforded for the counteraction of these deleterious effects. He showed how thus resulted both epidemic and zymotic diseases, the presence or absence of which in any locality, and the degree of their virulence depend on the sanitary surroundings. Cholera and dysentery are principally connected with the condition of the water supply; while an epidemic prevails the questions whether a given population shall suffer or escape may almost be predicted from a chemical analysis of the drinking water. It is to the physiologist and the chemist that we must look for the causes from which these baneful effects arise, and what are the conditions which should be altered to prevent or remove them. The engineer steps in after these causes have been pointed out, and it is for him to design the methods of prevention or removal.

From *Nature* 20, Oct. 30, 640, 641, 642; 1879.

articles

Relevance of Afar seismicity and volcanism to the mechanics of accreting plate boundaries

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The seismicity, the horizontal and vertical ground displacements, and the geometry of active faults and fissures during the seismic and volcanic events of November 1978 in Southern Afar are consistent with an accentuation of the pre-existing topography of the Ghoubbet-Asal rift. The zone of sizeable permanent deformation is restricted to several hundred square kilometres. Elastic rebound models seem to explain the observations. The events occurred in the vicinity of the suggested location of the tip of a lithospheric crack which may have been responsible for the progressive opening of the western Gulf of Aden.

AN important seismic swarm started on 6 November 1978 in the subaerial part of the rift which links Ghoubbet al Kharab to Lake Asal in the Republic of Djibouti (Fig. 1). North-west-south-east trending faults and fissures were reactivated in the inner floor zone of the rift, over a width of 3–4 km and visible length of 15 km, and a new volcano, Ardoukoba, came into being between 7 and 14 November. The whole sequence of events took place in an intensely studied section of the world ridge system, one of the few such sections that stand above water. This paper describes the seismic and tectonic aspects of the events and summarises data, much of which have been previously published only in French. We attempt to relate the events to the structural environment of Southern Afar and to test their relevance to geodynamical models of the Red Sea-Gulf of Aden plate boundary. More detailed accounts of the various observations and measurements will be published elsewhere.

Seismicity

The seismic swarm started on 6 November 1978, and lasted for 2 months, compared with only 1 week for the volcanic eruption¹. It was recorded by the seismological network of eight stations, most of which have been operating since 1972. Only three stations were operating at the beginning of the swarm, but all stations were back in use by 10 November. After a first increase in seismicity on 6 November when two earthquakes with $m_b > 4$

were recorded, the climax occurred on 7 and 8 November. On 7 November, 15 earthquakes had $m_b > 4$ and the major shock reached a m_b of 5.3. This event was located in the axial part of the Ghoubbet, not far from the coast (Fig. 1). On 8 November eight shocks with $m_b > 4$ and another one reaching $m_b = 5$ were recorded. From 7 to 10 November, the daily number of recorded events with $m_b > 0$ exceeded 2,000.

Most of the earthquakes that have been located so far occurred along the axial fault zone in the Ghoubbet; preliminary fault plane solutions suggest normal faulting, in agreement with observed submarine scarps. A composite fault plane solution from a series of earlier earthquakes gave a similar result (refs 2, 3 and Fig. 2). There was little seismic activity in the subaerial section of the rift where the volcanic eruption occurred. The main locus of seismicity migrated from west to east with time through the Ghoubbet straights to the Arta transform fault zone, following closely the active plate boundary whose detailed geometry is now reasonably well understood (refs 4–8 and Figs 1, 2). This eastward migration pattern is thus very similar to that observed in 1973 during a previous swarm in the Gulf of Tadjourah⁹.

Geodetic measurements and levelling

In 1972–73, a network of 22 geodetic stations was emplaced around the active Asal-Ghoubbet area¹⁰. Immediately following the November 1978 events nine stations were remeasured (Fig. 1). These measurements demonstrate that between 1973 and 1978 a large amount (at least 1.6 m) of extension occurred perpendicular to the rift axis. To establish the characteristic dimensions ('wavelength') of the deformed zone, a second set of measurements of further geodetic points was made: the additional data^{11,13}, together with the levelling data to be discussed shortly, show that the zone of the deformation does not greatly exceed the width of the inner rift (of the order of 10 km). Tarantola, Ruegg and Lépine¹⁴ have computed the principal directions of strain which are compatible with the observations. They find two narrow bands of contraction (strains of the order of 10^{-4}) that border the axial part of the rift (4 km) where north-east-south-west expansion dominates (with strains of the

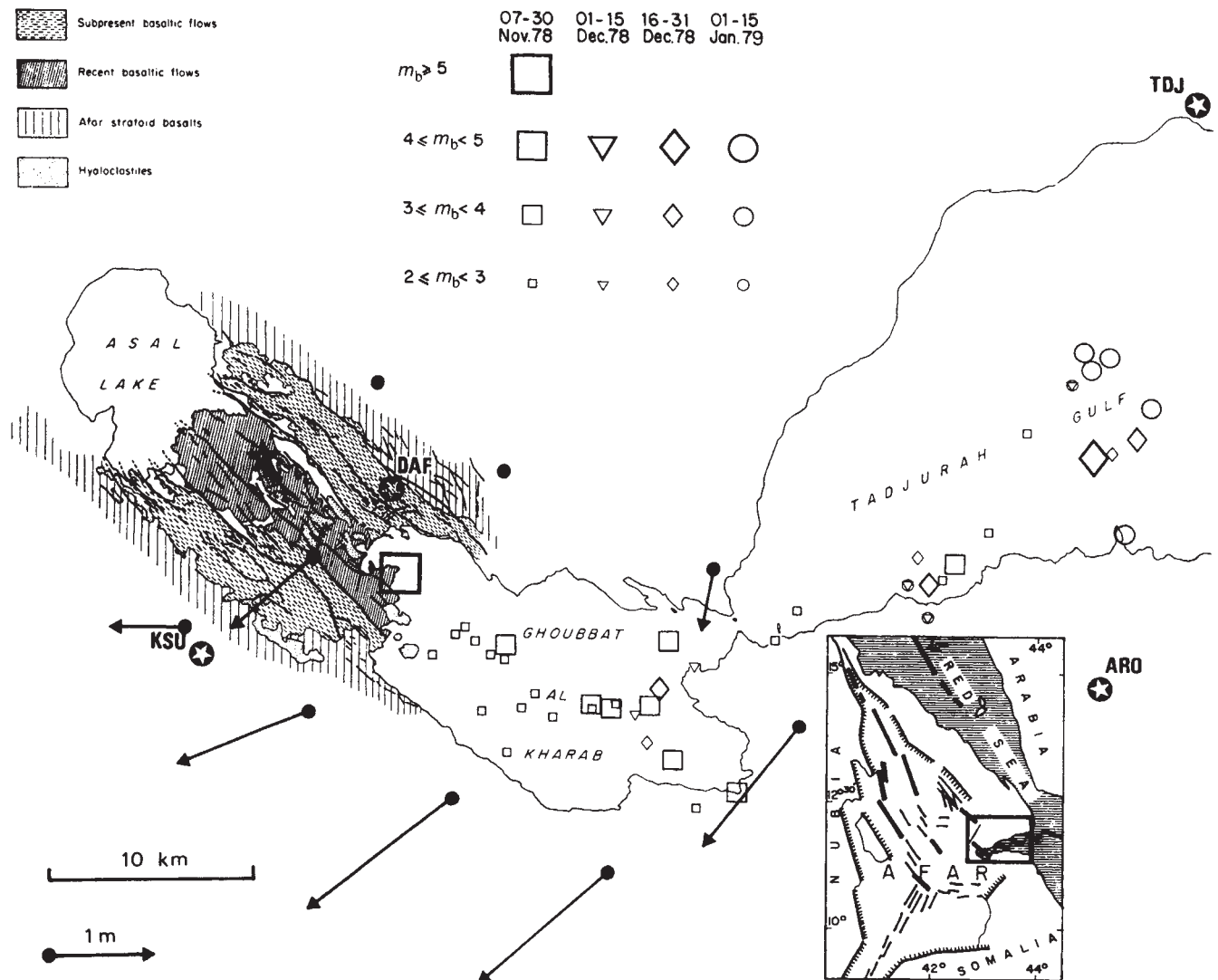


Fig. 1 Geological sketch of the Ghoubbet-Asal rift, evolution of seismicity from November 1978 to January 1979 (magnitude m_b and time of occurrence are given), and horizontal displacements (1973-78) measured at nine stations (the two northernmost stations are taken as reference, see refs 11, 12). Scales for displacements and for the geographical map are also shown. ☆, The November 1978 locus of volcanic activity; ⊙, some seismological stations. The general tectonic location is shown in the inset.

order of 2×10^{-4}). Computed strains seem to decrease with distance away from the Ghoubbet-Asal and become $< 10^{-5}$ ~ 10 km from the axis.

In addition to the geodetic measurements, releveling of a profile across the Ardoukoba rift, which had been measured first in 1972¹⁰ reveals that, in the central part of the inner floor, a zone 2 km wide and 4 km long has subsided more than 70 cm (Figs 3a and 4a). The amount of subsidence decreases towards Lake Asal and the Ardoukoba volcano, and also, although perhaps less rapidly, towards the Ghoubbet. Subsidence is not symmetrical with respect to the geometric axis of the rift, as a smaller rift-in-rift structure to the north is active, with a subsidence of its own of approximately 30 cm (RR in Fig. 4a). The general effect of the deformation seen in Fig. 4 is to accentuate the preexisting topography. Both flanks of the inner rift are elevated up to 20 cm over a 1 km width; these zones of elevation correspond roughly to the bands for which the geodetic data indicate contraction. Four km away from the rift axis, both to the north and to the south of it, the gradients of vertical displacement become much smaller. Actually, complementary levelling, although not yet fully processed, shows a broad uplift, some 20 km in wavelength, over which the narrow elevated shoulders and deep subsided central part are superimposed¹³.

Field tectonic observations

Detailed tectonic observations were made both on land and from air photographs at a scale of 1/10,000 (refs 15, 16). The recent structures, which seem to be related to the November events, generally follow older structures, with a north-west-south-east trend. Reactivation is clear in recent sediments (such as lacustrine limestones, diatomites and hyaloclastites) and in alluvial plains. Criteria such as freshness of the scarps, offsets in camel and road tracks or creeks have been used. However, it was not possible to find convincing criteria within the recent lava fields; such areas have therefore been left blank on Fig. 3a.

Structures fall into two categories: (1) normal faults, with a length from 100 m to several kilometres, and with a maximum throw of 70 cm (Fig. 5a); major faults are often associated with smaller antithetic faults. In alluvial zones, normal faults are sometimes paired to form graben structures (Fig. 5b) which may in some cases be the surface expression of deeper single faults. (2) Fissures of various types are also encountered: reopened fissures in which detrital filling has subsided (Fig. 5c), aligned collapse pits, which are interpreted as the surface expression of underlying fissures below the alluvial cover, and narrow and shallow small grabens that result from a coalescence of the

previous pits. Subaerial faults and fissures that have been reactivated during the events are very numerous and occur in a band of the inner rift which is 3–4 km wide and 8–10 km long (Fig. 3). Part of the deformation is concentrated in the small rift-in-rift structure which is located to the north-east of the inner floor (Figs 3 and 4b). The zone of present deformation is offset to the north with respect to the axis of symmetry of the overall rift topography. This suggests a northward migration or jump of the axial rift zone, consistent with the location of the present rift on the northern boundary of the main zone of Plio–Pleistocene lava accumulation and tectonics^{7,8,17,18}. There is also a suggestion of *en échelon* rifts, within the inner floor between Ghoubbet and Asal, which is supported by the patterns of seismicity in the eastern part of the Ghoubbet^{2,3,9}. This *en échelon* array is reminiscent (although the scale is quite different) of the *en échelon* fissure swarms which occur in the northern neovolcanic zone of Iceland^{19,20}. The three smaller *en échelon* fissures that fed the volcanic eruption opened in the northwestern part of the rift, in a region where surface tectonics and seismicity are not otherwise intense.

The vertical throw of faults is largest in the central section of the rift and decreases both towards Lake Asal and towards the Ghoubbet. Transverse tectonic sections were mapped in detail in December 1978 and are shown in Fig. 4b. There is a general agreement between the tectonic profiles and the levelling measurements (Fig. 4a), particularly for the southern half of the rift: for instance, two major reactivated scarps are crossed between levelling points 33 and 34^b (Fig. 3a) with a 70 cm downdrop. Tectonic profile 3, which is not far from the levelling route in this neighbourhood, shows a cumulated throw of the same order of magnitude (1 m). However, along the same tectonic profile 3, a discrepancy appears between levelling points 35ⁱ and 35ⁱⁱ: whereas the area is devoid of identifiable traces of brittle deformation (Fig. 3a), the levelling shows a 30 cm elevation difference (Fig. 4a). This may be because fresh brittle deformation is more difficult to observe in the lava surfaces which are found in this zone. Similar mismatches are found elsewhere, in a similar context. They may be related either to block tilting or to more distributed deformation of the surface cover in response to deep-seated faulting. Also, it is not certain that levelling and tectonic profiles reflect activity over the same time period.

Episodic rifting along accreting plate boundaries

There are interesting similarities between the observations of seismicity and tectonic deformation presented here and those reported by Björnsson *et al.*^{19,20} for a rifting episode on the plate boundary in the northern neovolcanic zone of Iceland. We have already pointed out the common *en échelon* arrangement of fault and fissure swarms, although in the Icelandic case only one such swarm has been reactivated since the current rifting episode started in 1975. A comparison between Figs 1 and 4 given here and Fig. 6 of the Björnsson *et al.* paper²⁰ reveals the striking similarity in horizontal and vertical ground movements in both cases. Note particularly the association of axial subsidence and extension with elevated side-shoulders showing evidence of contraction. This similarity between current rifting in Afar and Iceland (despite other differences) suggests that we are witnessing the fundamental mode of deformation at the axis of oceanic ridges. This adds to the already vast set of observations suggesting that there are few qualitative differences between tectonic processes in the axial zone of oceanic ridges and those which can easily be observed directly in Afar and Iceland^{21–24}.

One should not misinterpret¹ the relationship that may exist between a single event and long term motions occurring at a plate boundary. It is the superimposition of numerous discrete events of this kind both in space and time along the plate boundary that are integrated to give the slow (1 cm yr⁻¹) motion of the plates^{17,18,25–27}. Thus, the large extension of ~2 m that occurred between 1973 and 1978 in the Ghoubbet–Asal rift zone does not reflect the increase in distance between the Arabian and African plates in the same time interval.

Elastic rebound and faulting

Perhaps one of the most important results to emerge from the geodetic measurements is that deformation occurred in a coherent pattern, in an area that probably does not exceed a few tens of kilometres in width. The first model which comes to mind to account for these observed deformations is the elastic

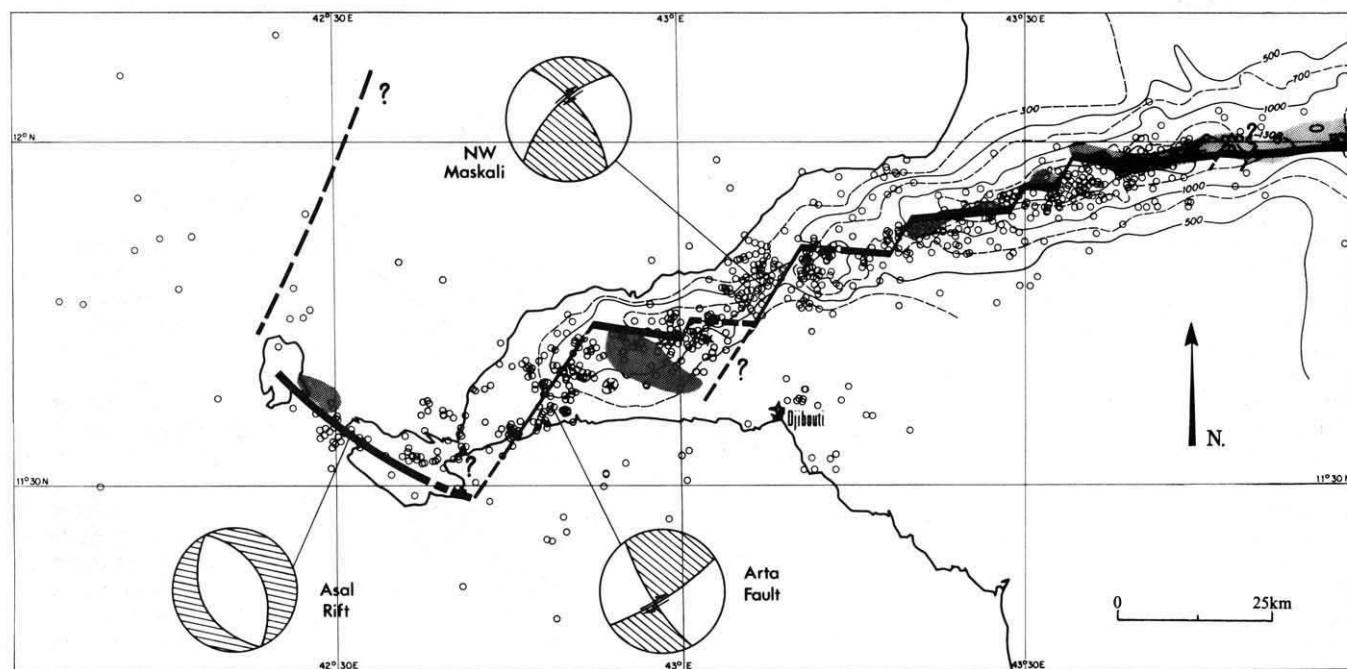


Fig. 2 Detailed bathymetry, earthquakes (○) and composite focal mechanisms for the period 1972–77 (see refs 2, 3, 17). ☆, Submarine volcanoes. Negative axial magnetic anomalies from Fig. 6 are shaded (after ref. 17).

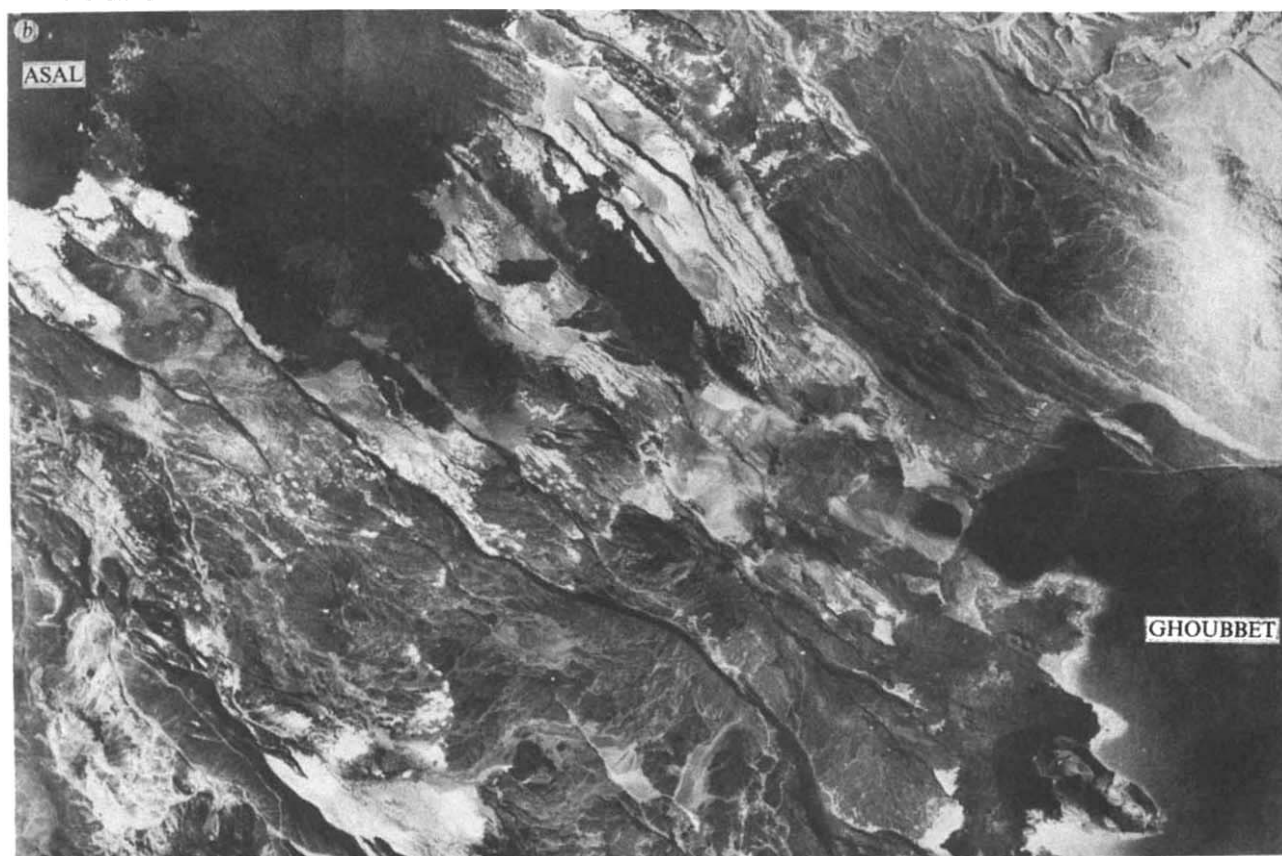
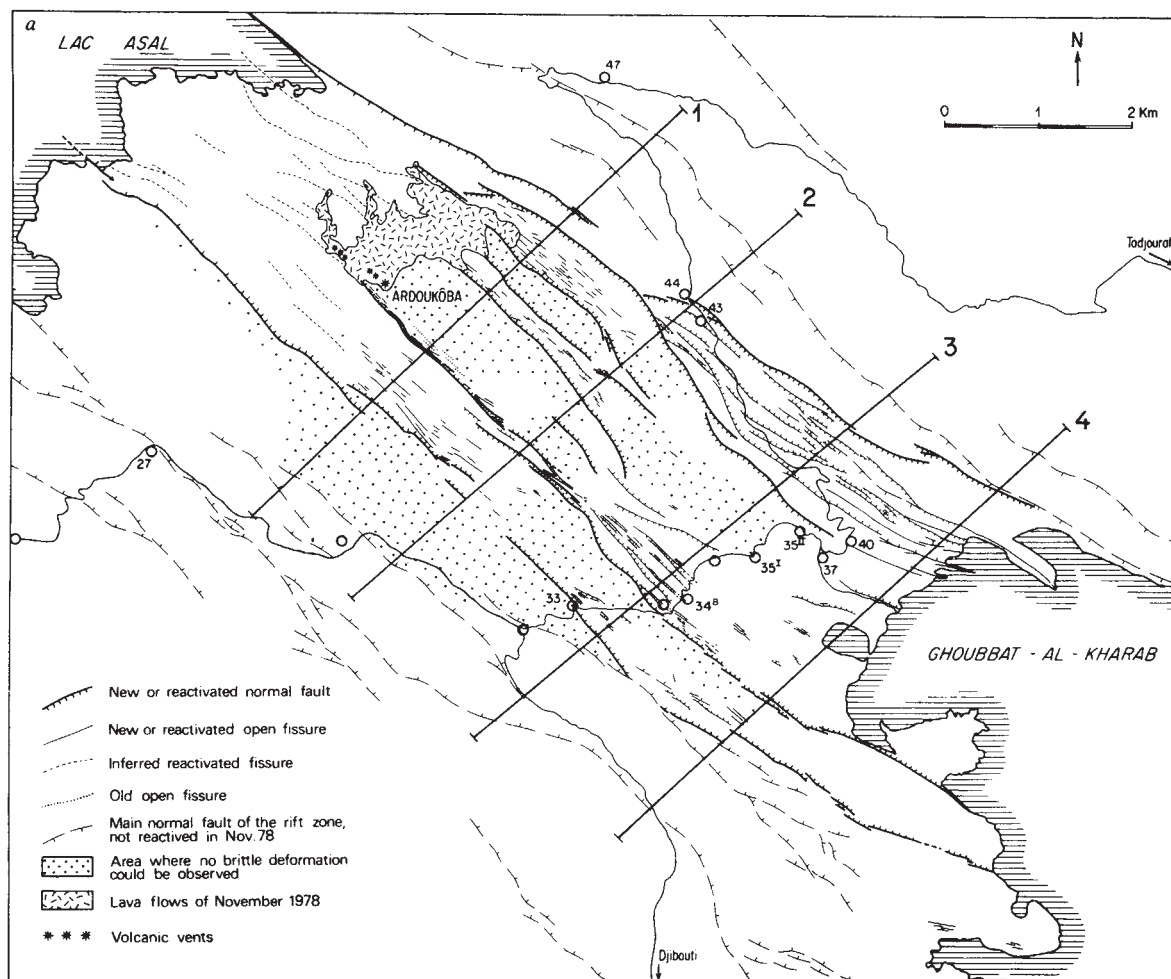


Fig. 3 *a*, Observed new or reactivated tectonic features in the Ghoubbet-Asal rift. Locations of tectonic profiles shown in Fig. 4*b* are given. The levelling route is also shown and several stations are labelled for reference. *b*, Composite air photograph of the same area at the same scale.

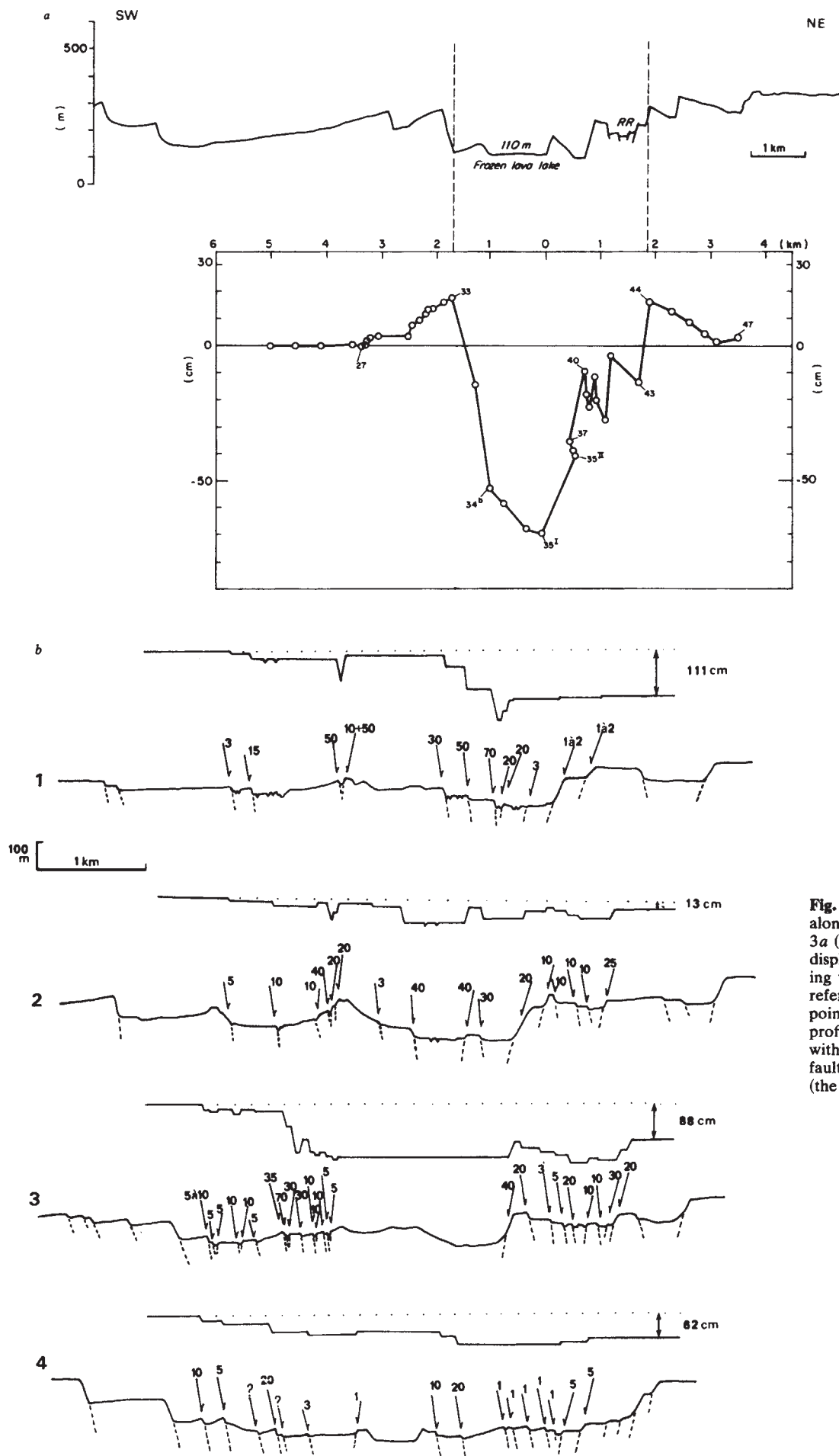


Fig. 4 a, Topographic profile along the levelling route of Fig. 3a (top) and measured vertical displacements (1973-78), taking the southernmost point as reference (bottom). Labeled points as in Fig. 3a. b, Tectonic profiles (locations in Fig. 3a) with observed recent throws on faults and cumulated throws (the southernmost point is taken as reference).

rebound model, introduced by Reid²⁸ for the study of the 1906 San Francisco earthquake. The model must, of course, be adapted to the case of normal faulting. The global forces that are responsible for plate motions presumably put the whole of Afar under extension with the principal direction being roughly north-east-south-west and energy is stored as elastic deformation builds up. During the seismic events, this energy is released and a zone of tens of kilometres in width is permanently deformed. Mansinha and Smylie²⁹ give analytical expressions which enable one to compute the displacement fields due to slip on inclined, finite, dip-slip (and strike-slip) faults, which are modelled as Volterra dislocations. Savage and Hastie applied this formula to the case of a fault with both dip- and strike-slip (refs 30, 31). Their results suggest that both vertical and horizontal ground displacements shown in Figs 1 and 3 can reasonably be accounted for by dip-slip faulting (with a throw of more than 1.5 m) on two normal faults with opposite dips, corresponding roughly to the walls of the inner rift. This would especially agree with the observation of both elevated and contracted lateral bands (see, for instance, Fig. 8 of ref. 30). Of course, faulting did not necessarily occur instantaneously on both faults, and these two faults give an integrated picture of the action of many smaller faults during the November 1978 swarm. This deformation is to be added to the deformation that may have taken place in the previous years. Alternatively, the horizontal displacement data can be accounted for by a 3-m opening of two finite vertical fissures, trending north-west-

south-east, close to the axis of the inner rift, one in Ardoukoba rift and the second below the Ghoubbet waters where most of the seismicity occurred¹⁴.

We should note at this point that much of the permanent outward tilt of blocks on both sides of the rift may be due to the integration of such deformation episodes and not only to isostatic response²¹. This may help to explain observations of rather large tilts on oceanic ridges.

1978 Events and geodynamical models of Afar

Although various plate tectonic analyses of the Red Sea-Afar-Gulf of Aden area have been published^{18,25} it is still apparently very difficult to define plate boundaries within Afar^{7,8,17}. On the basis of a new detailed aeromagnetic survey of the Republic of Djibouti (Fig. 6 and refs 7, 8), one of us (V.C.) has proposed that the western termination of the Gulf of Aden and the Gulf of Tadjourah were opened by the westward propagation of a crack in the lithosphere. Magnetic data and rock ages on land are interpreted to show that the crack has been propagating fairly regularly at $\sim 3 \text{ cm yr}^{-1}$. It is suggested that the tip of the crack is now lying in the vicinity of Lake Asal. Bathymetric data and seismicity recorded over the period 1972-78 (refs 2, 3, 9) agree with this suggestion (Fig. 2): in particular, Lake Asal is seen to mark the westward termination of the narrow, well defined band of seismicity which corresponds to the active plate boundary.

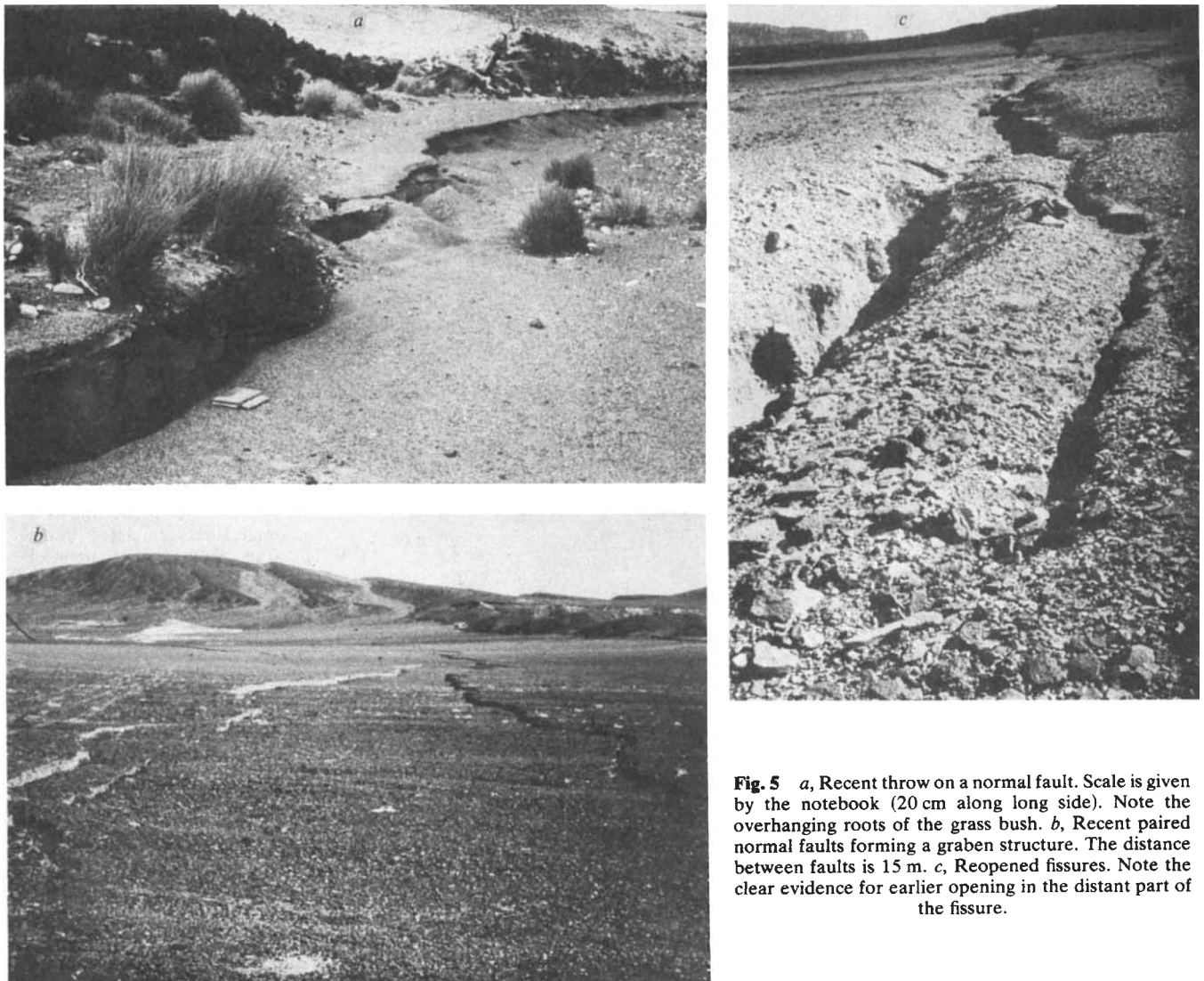


Fig. 5 *a*, Recent throw on a normal fault. Scale is given by the notebook (20 cm along long side). Note the overhanging roots of the grass bush. *b*, Recent paired normal faults forming a graben structure. The distance between faults is 15 m. *c*, Reopened fissures. Note the clear evidence for earlier opening in the distant part of the fissure.

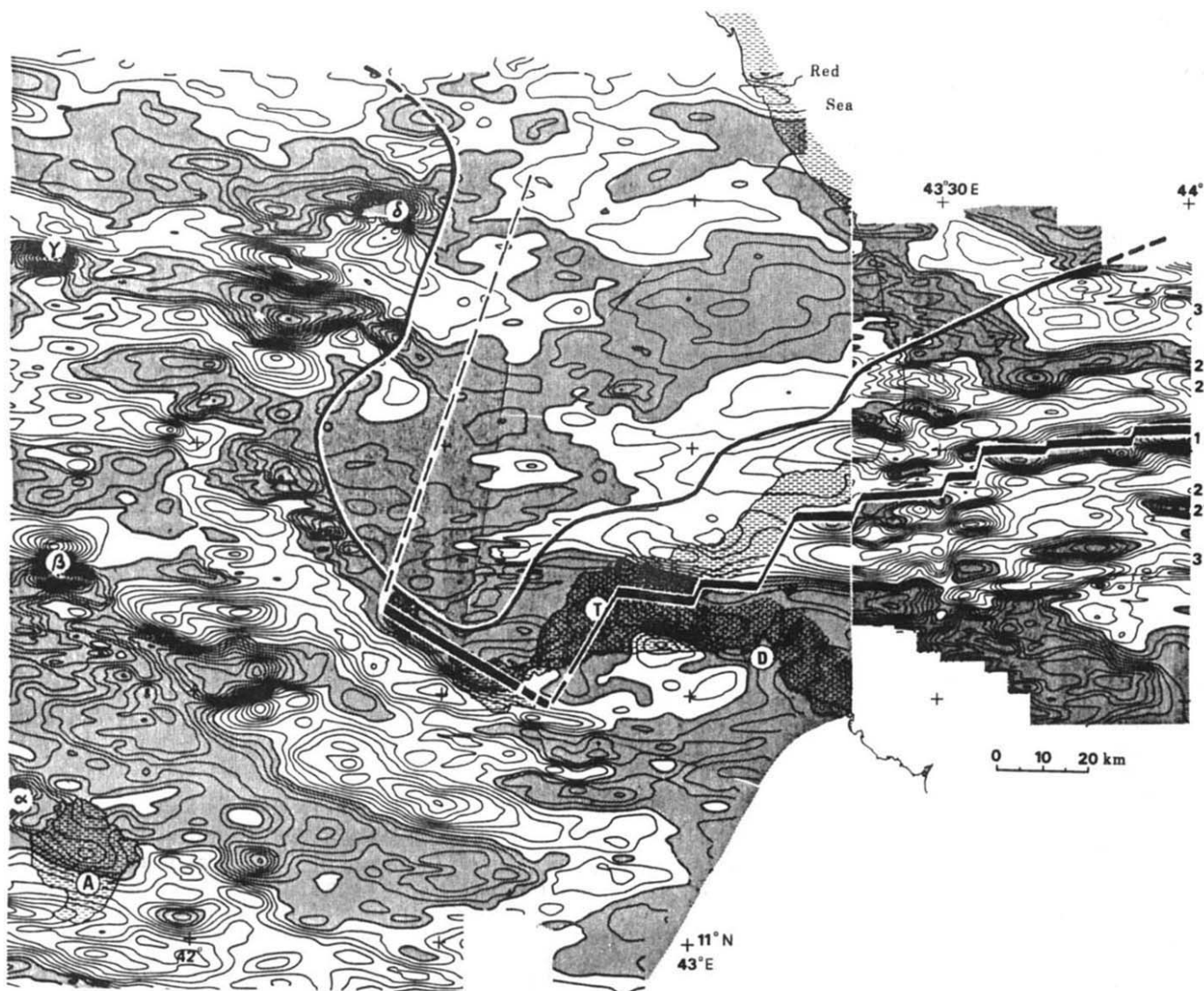


Fig. 6 Aeromagnetic anomaly map of the Republic of Djibouti (after refs 7, 8, 17). The plate boundary is indicated as a sequence of thick (ridge) and thin (transform fault) segments; the Mak'arrasou "transform fault" (refs 4–6) is shown as a dashed line. Also shown as a sinuous line is the boundary between zones of oceanic and more subdued magnetic styles (see ref. 17).

West of Lake Asal, seismicity seems to be more sparse and diffuse.

Thus, the November 1978 events would have started close to the suggested location of the crack tip. It is interesting then that the migration of seismicity away from the crack tip during the swarm and the distribution of the amplitudes of deformation (which are larger in the Ghoubbet than in the immediate surroundings of the volcanic centre) are compatible with the idea that to the west of the crack tip lies a locked area which is more resistant to deformation than the zone along which the plate boundary has already penetrated.

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The eruption of Soufrière volcano, St Vincent April–June 1979

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The Soufrière volcano in St Vincent began to erupt on 13 April 1979 after 10 months of mild premonitory activity. A series of strong vertical explosions between 13 and 26 April generated ash falls, pyroclastic flows and mudflows. From about 3 May onwards basaltic-andesite lava has been accumulating in the summit crater.

ST VINCENT is in the south of the chain of volcanic islands which comprise the Lesser Antilles Island Arc (Fig. 1). The island is 30 km long and the Soufrière volcano constitutes the northernmost third of the island with a diameter of 11 km at sea level. It is a strato-volcano 1,220 m high with an open summit crater 1.6 km in diameter located in the southern part of a 2-km wide somma crater. The summit crater has been occupied by a 1-km wide crater lake throughout most of its history¹⁻⁴. Explosive eruptions occurred in 1718, 1812 and 1902–03; the 1902–03 eruption was preceded by a 14-month period of felt earthquakes, and generated ashfalls, mudflows and pyroclastic flows climaxing on the afternoon of 7 May, 1902, when pyroclastic flows swept down valleys leading to the east and west coasts killing 1,565 people. The eruption continued intermittently until March 1903 by which time the total volume of uncompacted pyroclastic ejecta is estimated to have been 0.42 km³ (ref. 5).

In contrast, the eruption of 1971–72^{4,6} was aseismic and non-explosive. Between October 1971 and March 1972, 80 × 10⁶ m³ of basaltic andesite lava were quietly extruded into the summit crater causing the evaporation of 55 × 10⁶ m³ of water out of an original lake volume of 75 × 10⁶ m³. During the later part of this eruption seismic activity at the volcano was monitored by three high-gain, short-period seismographs all within 4 km of the crater but no seismic events were recorded other than small surficial events associated with the growth of the lava mass. The number of these events had declined almost to zero by the time that the extrusion of lava ended in March 1972 and they had ceased entirely when the seismograph stations were withdrawn in August 1972.

Activity premonitory to the 1979 eruption

Since August 1972, the nearest seismograph station to the volcano which was in continuous operation was station SVT 19 km to the south. The temperature at the southern edge of the crater lake, which had reached a peak of 81.5 °C in November 1971 was measured twice per month and found to decline steadily until January 1975 when it stabilised between 23 and 26 °C which is 1–4 °C above ambient conditions. The crater lake was inspected about every 3 months. After March 1972, the crater lake and island showed little change other than a gradual erosion of loose material from the island. Fumarolic activity was at first widespread over the island but by 1975 activity was concentrated in a depression of diameter ~20 m in the centre of the island. A bathymetric survey of the crater lake was made in February 1973⁷ and surface temperature surveys were made in March 1974 and December 1978. In March 1974, a large yellow stain became visible on the surface of the lake in the south-

eastern corner. At this time the temperature around the edge of the lake ranged from 30 to 33 °C, rising to 47 °C close to the lava island. The yellow stain persisted until the start of the 1979 eruption. A similar stain was reported by Senn⁸ in 1946 after a series of local earthquakes had been felt in northern St Vincent.

The first signs of abnormal activity which might be related to the present eruption were observed between August and November 1976 when a phreatic eruption of the Soufrière volcano in Guadeloupe 310 km to the north was in progress. Between 11 August and 21 October the water temperature at the southern edge of the lake rose from 23 to 30.5 °C. No other signs of abnormal activity were observed and by 19 January 1977 the temperature had decreased to 25 °C. In February 1978 the temperature began to rise slowly and irregularly, reaching a peak of 31 °C on 21 August 1978. On 2 June 1978 a short-period seismograph linked by radio telemetry to the University of the West Indies in Trinidad was established at Belmont, 9 km south-west of the crater (SVB in Fig. 2) and on 12 June this station began to record events which were tentatively identified as B-type volcanic earthquakes⁹. The events occurred in bursts of up to 20 and their frequency increased throughout June and July reaching a peak on 30 July when 40 events were recognised. Because of the simultaneous rise in lake temperature and increase in seismic activity, the local authorities were advised on 24 August that an abnormal situation might be developing at the volcano. Tilt monitoring stations of the optical tiltmetry type which were first established on the eastern flanks of the volcano in February 1977 at distances of 3 km and 6 km from the crater were re-occupied on 7 March 1978 and again on 17 September 1978. The results showed apparent tilts of order 3–5 μrad, consistent with inflation of the volcano, between March 1977 and September 1978. These were the first optical tiltmetry measurements to be made in St Vincent and the standard error of a single set of measurements was of the order of 4 μrad. The tilt results alone could not therefore be taken as conclusive evidence that the volcano was inflating. Inspection of the crater lake and island showed no significant change but a second telemetered seismograph station established 3 km south-west of the crater on 19 September (SVV on Fig. 2) confirmed that the seismic events originated close to the volcano. From 19 September 1978 to 12 April 1979 these two stations continued to detect B-type events at rates of up to 50 per day but the rate of occurrence showed no tendency to increase or decrease with time. No earthquakes were reported to be felt and only a few of the stronger events were detected by station SVT. During the same period the surface temperature of the lake was measured weekly and fluctuated between 27 and 30 °C up to 11 April 1979 when it was 28 °C. On 24 December 1978 the surface temperature of the lake was measured on one complete circuit of the lake around the island and was found to be a uniform 30 °C except for a hot spot close to the eastern edge of the island where it was 47 °C.

At 07.13 UTC on 12 April a large B-type earthquake was recorded by SVV and SVB and at about the same time continuous tremor became recognisable above the background micro-seismic level. For the following 12 h B-type events continued at

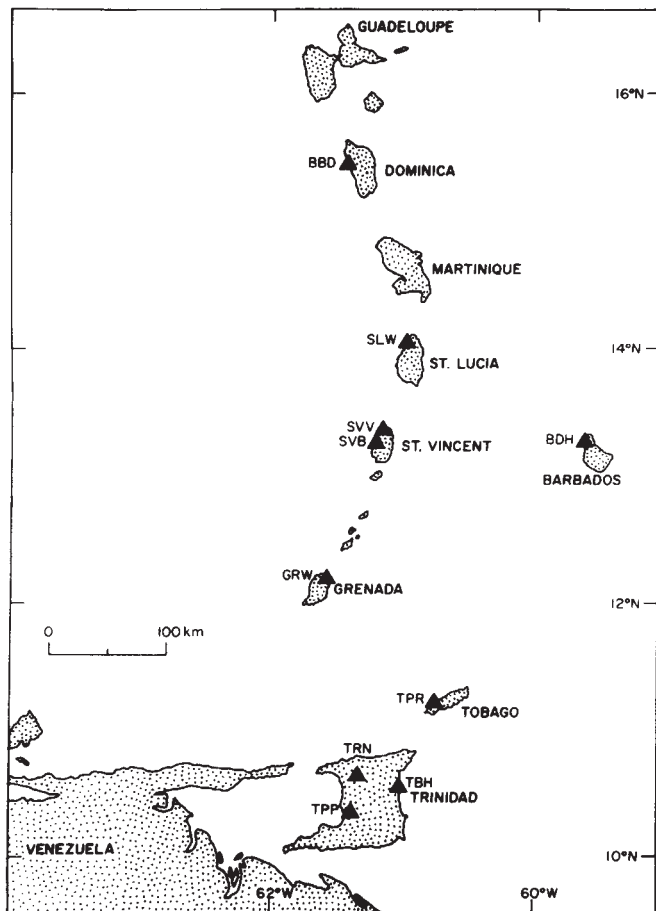


Fig. 1 Regional position of St Vincent in the Eastern Caribbean. The seismograph stations (▲) shown are linked by radio to Trinidad; many other instruments exist in the region.

a mean rate of about one per hour until 19.54 UTC when their frequency began to increase. Between 19.54 and 02.04 UTC on 13 April, 38 events were recorded with generally increasing amplitude and the level of continuous tremor increased steadily. Between 02.04 and 04.30 UTC, 70 events were recorded before continuous tremor saturated both instruments and individual events could no longer be distinguished. At 03.30 UTC (11.30 p.m. on 12 April local time) the government of St Vincent was warned that a highly abnormal situation existed at the volcano, but local residents 6 km east of the crater reported that they had neither seen nor felt anything unusual up to this time. At about 08.00 UTC loud rumbles were heard both east and west of the volcano and shortly afterwards fine ash began to fall at distances of up to 5 km south-east and south-west of the crater. When dawn broke at about 09.30 UTC the volcano was seen to be erupting a column of steam and ash and evacuation of the areas north of the Rabacca and Wallibou rivers (Fig. 2) began immediately. The evacuation was completed smoothly by 20.00 UTC and within the next 24 h about 15,000 people were evacuated from this area.

Early stages of the eruption

Figure 3 summarises the eruptive and associated activity from 12 April to 17 June. From 09.30 to 15.00 UTC on 13 April no one person kept the volcano under continuous observation but from numerous reports we deduce that there was continuous strong steam emission from the crater for the whole of this period, punctuated by powerful vertical explosions which projected ash-laden clouds to heights in excess of 8 km. After the initial outburst further distinct explosive phases were observed at about 10.15 and 15.15 UTC. At 11.20 UTC the eruption column was observed from a commercial aircraft to be at a height of 10 km, 150 km, south-east of the volcano. The column

height was then ~8 km and an anvil-shaped cloud extended 80 km to the east. The column was again seen at 12.50 UTC from the same distance and appeared to be unchanged. At 15.00 UTC two of us saw the column from an aircraft approaching St Vincent from the south. When first observed the eruption column and cloud fitted the earlier description, but at 15.15 UTC a dark, cauliflower-shaped cloud was projected upwards above the column and drifted off to the east. At 16.00 UTC we flew low over the eastern and western flanks of the volcano in a light aeroplane and landed briefly at the Rabacca River 7 km south-east of the crater. At this location, fine light-grey volcanic ash was falling lightly but steadily and had formed a layer ~3 mm thick. The aerial inspection showed that a thin layer of ash had been deposited to distances of at least 10 km from the crater. We saw no evidence of pyroclastic flow deposits on the lower flanks, but the uppermost third of the volcano was obscured by falling ash.

After 16.00 UTC the dense steam-and-ash column dispersed although steam continued to pour from the crater. At 19.30 UTC we established an observation post at Belmont (SVB on Fig. 2) and since then the volcano has been kept under continuous visual surveillance. Explosive activity was renewed at 20.05 UTC and further explosive phases began at 21.08 UTC and 23.50 UTC on 13 April and at 01.08, 07.06 and 15.50 UTC on 14 April. The explosive phases varied in duration and intensity but were all broadly similar. Each began with rumbles audible from Belmont followed within 1 min by the emission of a white cloud which rose and expanded rapidly. One to two minutes later the cloud became much darker and assumed a distinctive cauliflower shape, rising and expanding rapidly and mushrooming outwards at a height of about 4 km above the crater rim. Ultimate heights of the eruption columns could not easily be estimated from the ground but the eruption clouds generated by the explosions from 21.08 UTC on 13 April onwards were photographed from a NOAA Earth satellite and maximum column heights have been estimated by comparing the temperature at the top of the cloud with standard atmospheric temperatures. By this method the columns from the explosions at 21.08 UTC on 13 April and at 01.08 and 15.50 UTC on 14 April have been estimated to reach heights of 17–18 km. The other eruption columns did not penetrate the meteorological cloud layer and no similar estimates of their heights could be made. The lower part of each cloud drifted to the west in the prevailing trade winds while the upper part drifted to the east in the upper-level westerlies. Transport of the ash-plume resulted in ash fall on Barbados, 180 km to the east on the afternoons of 13 and 14 April. On the western flanks of the volcano air-fall from this series of explosions consisted mainly of fine, light-grey, crystal-rich ash but fresh scoria up to 70 mm in diameter fell on Georgetown, 9 km south-east of the crater. The maximum air-fall thickness at the eastern flank of the volcano 7 km from the crater was 40 mm. All explosive activity originated in the western part of the crater at the approximate location of the island formed in 1971–72.

Pyroclastic flows

During the explosive phase beginning at 21.08 UTC on 13 April we observed a pyroclastic flow which descended the upper part of the Wallibou river valley to the south of the crater. At the Rabacca river crossing 7 km south-east of the crater it was reported that a pyroclastic flow descended part of the way down the Rabacca river valley during the same explosive phase. Further signs that pyroclastic flows were being generated during the phase beginning at 01.08 UTC occurred on 14 April when incandescent material was projected over the crater rim and cascaded down the southern and western flanks. A low-level aerial reconnaissance at dawn (about 10.00 UTC) on 14 April showed a fresh pyroclastic flow deposit in the upper part of the Rabacca valley. The largest pyroclastic flow that we observed descended the 3-km long Larikai valley to the west of the crater at 16.00 UTC on 14 April and continued out to sea for at least 10 km. When inspected on site, 28 h after emplacement, the

deposit at the mouth of the Larikai was 1.5 m thick, ~250 m wide and contained scoria blocks up to 600 mm in diameter in a matrix of ash and lapilli. A pyroclastic flow rich in blocks of scoria and carrying trunks and limbs of trees descended the adjacent Roseau valley at about the same time, to within 100 m of the sea.

Subsequent explosive activity

From 17.00 UTC on 14 April to 20.57 UTC on 17 April the volcano was in a state of mildly explosive activity emitting mainly steam but with occasional cauliflower-shaped, ash-laden clouds rising to heights of 1–2 km above the crater rim. On 17 April the lower tilt-meter sites on the eastern flanks were reoccupied and showed tilts of ~4–6 μ rad indicating deflation of the volcano since September 1978. At 20.57 UTC on the same day another explosive phase similar to the previous ones began. Pyroclastic flows were again observed to descend the Larikai, Roseau and Wallibou valleys to the west of the crater but none reached the sea. The height of the eruption column was measured from a NASA aircraft near St Vincent and was

estimated to be 18.7 km. Ash fall at Belmont was coarser than in previous explosions. Initially it consisted of lapilli up to 10 mm in diameter grading down to fine ash over a period of ~50 min. Coarse scoria again fell on the eastern flanks as far as Georgetown. All these explosions were accompanied by severe electrical storms in which lightning discharges both within the eruption cloud and from cloud to ground occurred at a rate of more than 1 per s. Lightning strikes were observed at distances of at least 9 km from the crater and caused damage to trees 6 km east of the crater. A striking feature observed during the explosion of 17 April was the development of smooth, funnel-shaped bands of condensation around the eruption column immediately below the cauliflower-shaped eruption cloud.

After the 17 April explosion the volcano returned to a state of intermittent, mild explosive activity, generally declining in intensity but punctuated by two further explosive phases beginning at 10.37 UTC on 22 April and 03.53 UTC on 26 April. During the 22 April explosion a small pyroclastic flow descended the Larikai valley. A few minutes later a dilute pyroclastic flow was formed from ash that fell from a billowing cloud of

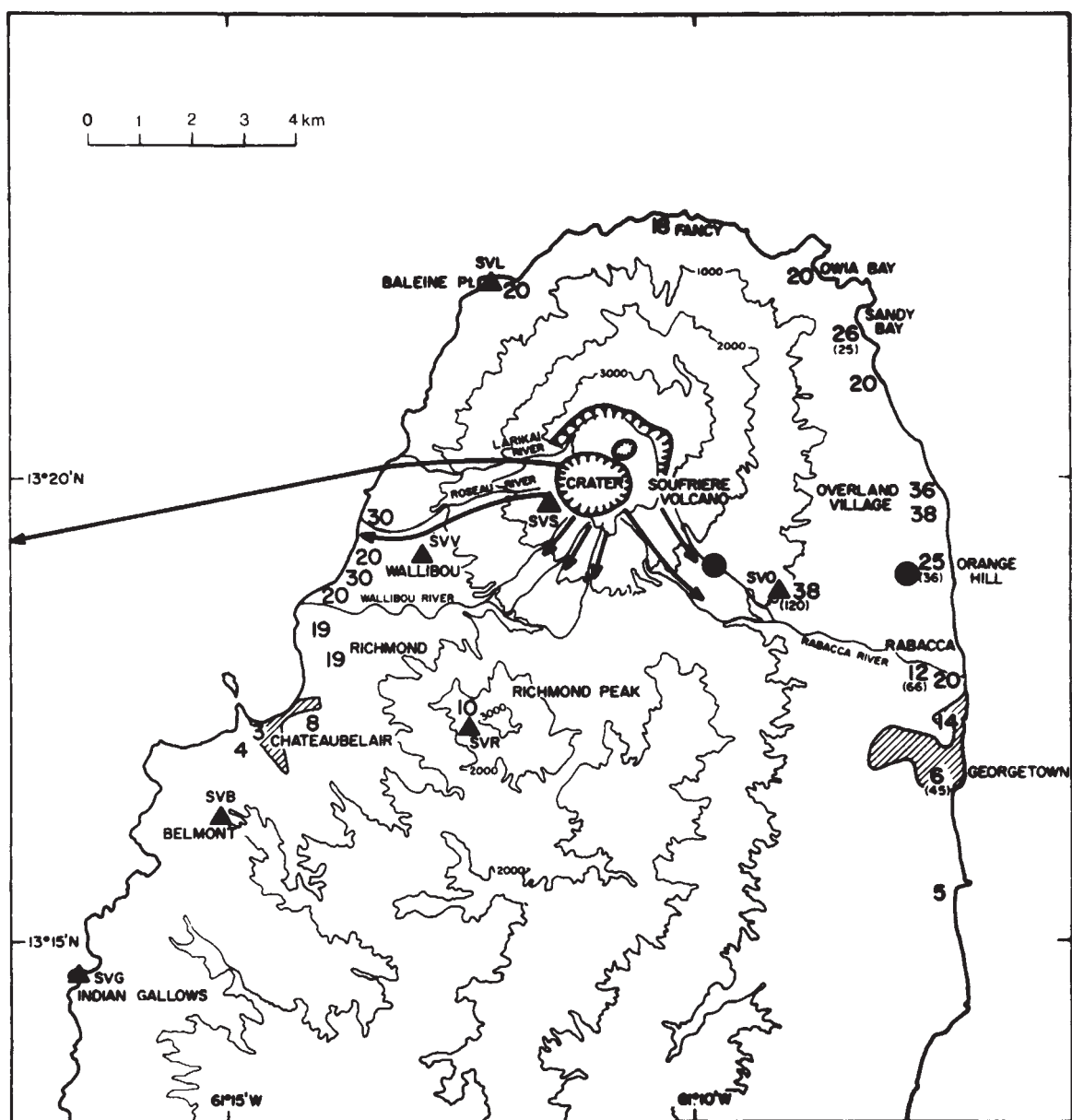


Fig. 2 The northern part of St Vincent island showing the location of the Soufrière volcano, major streams, general topography and two principal towns. The courses of pyroclastic flows known to have taken place in the 1979 eruption are shown as arrows and measurements of ash thicknesses (mm) are given as large numerals. The positions of tiltmeter (●) and seismograph station (▲) are also indicated. Figures in parentheses are maximum bomb dimensions (mm).

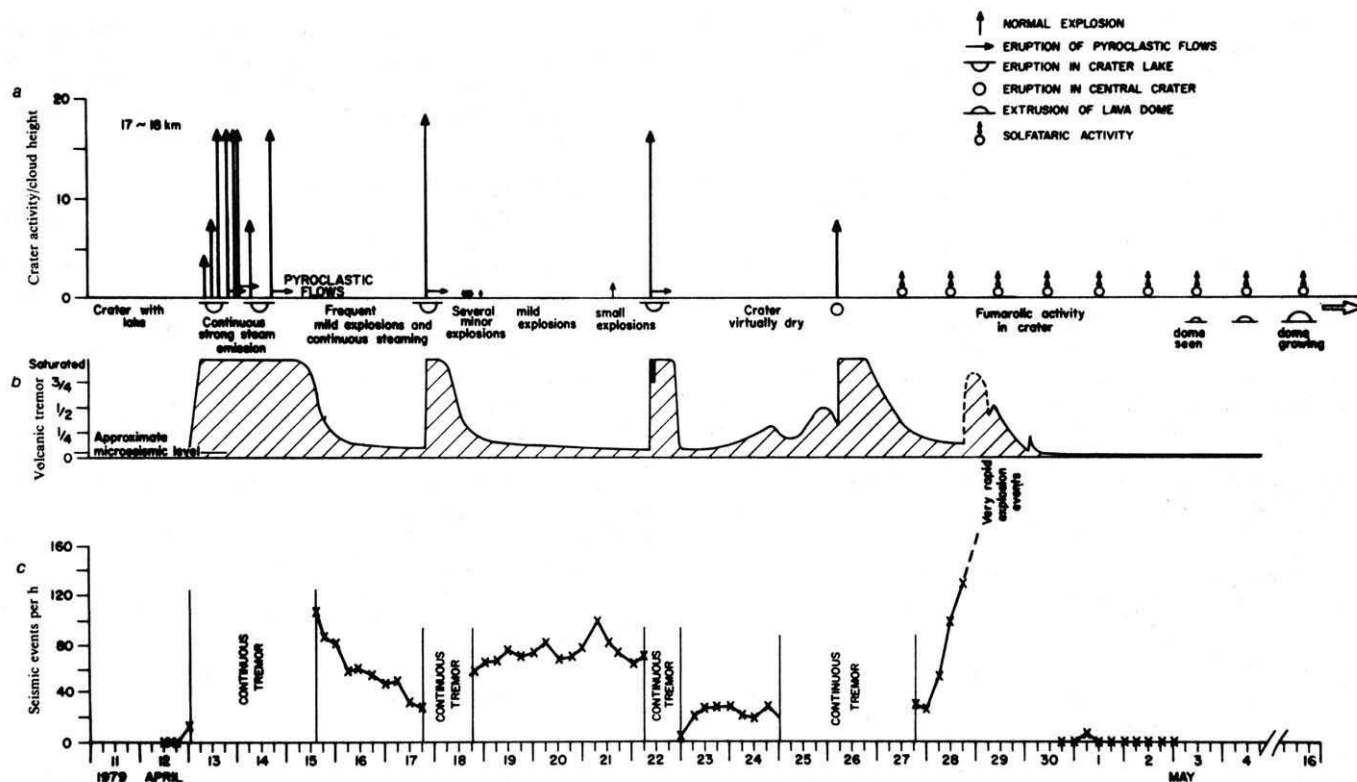


Fig. 3 Chronological summary of the major eruptive events and seismic behaviour of the Soufrière Volcano for the period 11 April–16 May 1979. *a*, The form of activity at the crater; *b*, the approximate level of continuous volcanic tremor; and *c*, the number per hour of discrete seismic events (explosion and B-type earthquakes), when these could be discerned from the continuous tremor.

airborne tephra and this flow, dark grey to black in colour, crossed the coast and spread as far south as Chateaubelair Bay. After this explosion the main axis of ash fall extended east from the crater with maximum total thicknesses along the east coast of the order of 40 mm at Overland Village, thinning to 10 mm at 5 km to the north and south. These figures are lower limits since each ash fall was rapidly eroded by wind and rain soon after deposition. Similarly the pyroclastic flow deposits in river valleys were rapidly reworked by streams and covered by mudflows which began to form on the night of 14–15 April. The final explosion of the series began at 03.53 UTC on 26 April. The height of the eruption column was measured from HMS Gurkha 15 km to the west and was estimated to be 8 km. Wind carried the eruption cloud from this explosion due south and deposited up to 4 mm of ash on Kingstown the capital.

The crater during the eruption

Throughout the explosive period frequent low-level reconnaissance flights were made over the volcano but the continuous steam emission combined with persistent low-level cloud prevented us from seeing into the crater until 17 April at about 13.00 UTC, when a brief view was obtained of the interior of the crater. The 1971 island was still largely intact except to the north where it was breached by a hole approximately 100 m in diameter connected to the crater lake. It was not possible to determine the extent or depth of the crater lake. On 21 April the first clear photographs of the interior of the crater were obtained. About one-quarter of the 1971 island remained, forming a horseshoe shape around a steaming vent. South and east of the island remnant, the floor of the crater was covered with mud and other debris but a portion of the crater lake remained in the northern and western parts, connected to the vent by a breach in the northern part of the island. Persistent heavy cloud at the summit then made aerial reconnaissance extremely difficult but on 30 April conditions improved and it was possible to see that only a fragment of the island remained. Most of the floor was covered with mud around a strongly steaming central depression. On 3 May the presence of a small

dome-shaped object in the eastern crater rim was reported to be steaming vigorously. Aerial reconnaissance on 7 May confirmed the presence of a fresh, black, dome-shaped mass of lava ~200 m in diameter rising to ~30 m above the crater floor. The crater itself was infilled with debris to above the level of the pre-eruption lake surface and was apparently dry except for a small pond abutting the northern edge of the new lava mass. Strong steam emission was taking place at the western edge of the lava and at several other places on the crater floor. The lava itself was draped with sediments which eroded rapidly over the next two days. Further aerial inspections were on 8, 9 and 11 May during which time the lava mass increased in size by about 40%. Between 14 and 16 May the lava mass was surveyed from the crater rim and from the crater floor. At this time it was approximately elliptical in plan with major axes 400 m from north to south and 300 m from east to west. Its sides sloped upwards at an angle of about 45° to a flat top with a slight depression in the centre. A small crescent-shaped pond abutted the lava on the north side while to the south-west and south-east two relics of the 1971 island protruded through the new crater floor. During the next month the lava continued to grow, mainly by lateral spreading over the crater floor, at a rate estimated to be $0.5\text{--}1 \times 10^6 \text{ m}^3 \text{ d}^{-1}$. Growth was most rapid to the north and north-east and least rapid to the south and south-east where the remnants of the 1971 lava restricted lateral spreading. The depression on the top of the lava gradually disappeared to be replaced by a slight rise from which several low ridges radiated. On 18 June the diameter of the lava was 715 m from east to west and about 700 m from north to south. Its height was estimated to be $110 \pm 10 \text{ m}$ and its total volume of order $25 \times 10^6 \text{ m}^3$.

Seismological observations

Three short-period vertical-component seismographs were in operation in St Vincent at the beginning of the eruption. Two of these (SVV and SVB in Fig. 2) were linked by radio telemetry to Trinidad where the signals were displayed visually on drum recorders and simultaneously recorded on magnetic tape for later analysis. The third station (SVT) operated with a standard

Table 1 Major oxide analyses of Soufrière samples

Component	Grey glass*	Dark glass*	Scoria block†	Composite ash fall‡	Lava flow§
SiO ₂	65.9	57.9	54.9	55.5	55.8
Al ₂ O ₃	13.7	14.3	18.7	19.2	18.4
FeO	6.77	9.38	8.23	7.43	8.58
MgO	1.23	2.86	4.08	3.44	4.05
CaO	4.05	6.34	8.94	8.70	9.11
Na ₂ O	3.34	4.02	3.35	3.43	3.32
K ₂ O	1.56	1.11	0.56	0.58	0.56
TiO ₂	1.08	1.64	1.01	0.97	1.05
P ₂ O ₅	—	—	0.12	0.12	0.11
Total	97.63	97.55	99.89	99.37	100.98

The modal analysis of air-fall ash collected at Grantley Adams Airport, Barbados, 14 April 1979 gave: crystals, 43.5%; grey glass, 33.7%; dark glass, 8.7%; lithics, 13.3%; carbonate and sulphate, <1.0.

* Barbados ash fall, 14 April 1979.

† Larikai pyroclastic flow, 14 April 1979.

‡ Belmont, 17 April 1979.

§ New analysis of sample from lava flow, 13 December 1971.

|| Total iron oxides reported as FeO.

photographic recorder in St Vincent. On 14 April, a second magnetic tape recorder and visual monitor were set up at Belmont. Initially signals were recorded from SVV, SVB and from SLW, 80 km to the north in St Lucia, BDH, 170 km to the east in Barbados, and BBD, 250 km to the north in Dominica. Other seismograph stations, monitored in Trinidad, were in Grenada, 130 km south of St Vincent and in Trinidad and Tobago. Throughout the eruption the larger network recorded only regional seismic activity at a level known to be normal for this region. In the vicinity of St Vincent the network has a detection capability for earthquakes down to about magnitude (m_b) 2.5, and no earthquake above this magnitude originated within 100 km of the Soufrière throughout the eruption. Between 15 and 21 April four more radio-linked stations were added to the network in St Vincent. On 28 April SVT was linked in to the system and on 12 May a station was established on the crater rim. The final station configuration is shown in Fig. 2. From the time that stations SVV and SVB became saturated by continuous tremor at 04.30 UTC on 13 April they remained saturated until about 13.00 UTC on 15 April (for the entire early period of explosive activity). At about this time the level of continuous tremor had declined sufficiently for individual events to be recognisable, and since then the numbers of events have fluctuated as shown in Fig. 3. A more thorough analysis of these events will be discussed elsewhere but, in the terminology of Minakami⁹, they are of two types—explosion-type earthquakes and B-type earthquakes. The relative time control between the stations of the network is $\sim \pm 0.02$ s but the continuous background tremor throughout the eruption has made it difficult to identify first onsets to better than 0.1 s. The pattern of arrival times at stations in St Vincent makes it clear that all the events originated within 1 km of the crater, but the emergent nature of the onsets and the station distribution (particularly the lack of a station very close to the crater rim) make it impossible to estimate focal depths by conventional first-arrival techniques to a precision better than ± 5 km. A reasonably good correlation was established between some of the larger explosion-type events and actual explosions visible at the crater. Both types of event were propagated with very low phase velocities and attenuated rapidly with distance which suggests they were of extremely shallow origin.

Perhaps the most interesting seismological feature of the eruption has been the continuous tremor. Apart from the initial 57-h period of very strong tremor, there have been several subsequent periods of tremor (Fig. 3). Each major explosive outburst has been accompanied by strong tremor, but no clear premonitory pattern emerged by which we could predict the onset of individual explosions by more than a few minutes. No

earthquakes have been reported to be felt during the eruption, but several people who were within 10 km of the volcano on the morning of 13 April reported feeling continuous tremor, and the tremor accompanying the 17 April explosion was reported to be felt at Georgetown.

Petrography and analysis of ejecta

Thin sections of fresh material from bombs and pyroclastic flow deposits show that plagioclase phenocrysts with oscillatory-zoned rims and shadow-zoned cores are the main crystalline phases. Some crystals, the largest of which are 10–12 mm long, contain gas bubbles and inclusions of olivine and glass. Other phenocrysts are pale yellowish-green clinopyroxene with rare and almost colourless orthopyroxene. Accessory amounts of subrounded olivine as well as euhedral amphibole and disseminated magnetite are also present. Fine-grained gabbroic cumulate inclusions 5–20 mm in diameter are common in many of the blocks and bombs. The matrix is glassy to microcrystalline. Tephra collected at Grantley Adams Airport, Barbados, on 14 April 1979 were analysed at the University of Rhode Island and the glasses from the same ashes were analysed by electron microprobe. The results are shown in Table 1. Whole-rock analyses of scoria collected from the 14 April Larikai pyroclast flow and of a composite scoria and ash fall at Belmont on 17 April were carried out at the Smithsonian Institution by electron microprobe. A sample from the 1971 lava was also analysed on the same instrument and all the results are shown in Table 1. The tephra produced in the eruption was found to contain two glass populations of dacitic and andesitic composition. The occurrence of banded scoria blocks in the new air-fall deposits on St Vincent confirm that the 1979 event was a mixed-magma eruption.

Comments

A full assessment of the size and significance of this eruption, the most intensively ever monitored in the Lesser Antilles, cannot be made until all the data have been analysed or indeed until the eruption comes to a definite end. Since 13 April, samples have been collected of the ejecta from each major explosion and the extent and thickness of these deposits have been constantly assessed. After each explosion the air-fall deposits other than those on the uppermost slopes had almost completely vanished within 48 h and the deposits on the volcano itself were partially reworked by mudflows during the same period. These observations illustrate the need for rapid sampling and also suggest that eruptions of the Soufrière which are of great significance from the point of view of public safety may leave little or no permanent stratigraphic record. Several tens of thousands of volcanic earthquakes, as well as many hours of continuous tremor, have been recorded on magnetic tape and most of the individual explosion since 15.15 UTC on 13 April have been extensively photographed, and the explosion of 17 April was particularly well recorded. The air-fall ash thicknesses of Fig. 2 are generally 1–2 orders of magnitude less than the thicknesses reported from the same locations after the explosions of 7–8 May 1902. Similarly the pyroclastic flows seem to have travelled much shorter distances than in 1902 when pyroclastic flows frequently reached the sea both east and west of the crater. Judging by these rough criteria we estimate that the total volume of pyroclastic ejecta in 1979 has been one or two orders of magnitude less than in 1902.

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Plio-Pleistocene environments at Gadeb prehistoric site, Ethiopia

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Widespread artefact-bearing gravels overlie Pliocene diatomites in the plain of Gadeb ($7^{\circ}02' - 7^{\circ}13'N$, $39^{\circ}15' - 39^{\circ}28'E$) at 2,300 m in east-central Ethiopia. The diatomites are from 2.7 to 2.35 Myr old, and the bulk of the Developed Oldowan and Acheulian tools are between 1.5 and (?) 0.7 Myr old. Drying out of the Pliocene lake, deposition of the gravels, and erosion of the Webi Shebele gorge reflect the interplay of volcano-tectonic and climatic events, as does the biostratigraphy of the Pliocene diatomites.

A PROBLEM for prehistorians and earth scientists working in East Africa is how to distinguish depositional changes in lakes and river valleys which stem from climatic oscillations from those related to volcanic and tectonic activity¹⁻⁴. A further difficulty near the African Rift Valleys is that much of the older field evidence has been faulted, tilted, removed by erosion, or buried so that conflicting interpretations of the age and significance of Plio-Pleistocene deposits in East Africa make it difficult to reconstruct prehistoric environments during this critical period in hominid evolution with appropriate precision⁵⁻⁸. Accordingly there is a real need to seek out areas in Africa in which complete sequences of late Cenozoic deposits have been preserved from erosion and tectonic disruption, and in which evolutionary changes in the plants and animals may be related to prehistoric cultural changes, set within a radiometrically dated time scale. Recently mapped and excavated fluviolacustrine deposits at 2,300 m on the plain of Gadeb, near the headwaters of the Webi Shebele River, in the volcanic uplands of east-central Ethiopia (Fig. 1), seem to fulfil these prerequisites, and may well become a stratigraphic datum for the rest of East Africa.

At Gadeb, artefact-bearing fluviatile sands and gravels overlie Pliocene lacustrine diatomites and clays (Fig. 2). The fluviatile sediments occupy erosional channels, and postdate a 2.35 ± 0.2 Myr old welded tuff which overlies the lake deposits. A marked erosional unconformity separates the lacustrine and fluviatile series.

The stone implements associated with the fluviatile sediments are manufactured mostly of local basalt and welded tuff, and belong to the Developed Oldowan and Acheulian traditions^{9,10}. The occasional obsidian tools must, on present knowledge, have come from sources in the Rift some 100 km away (W. H. Morton, personal communication). Most, but not all, of the artefact-bearing sediments postdate a 1.5 Myr pumice deposit, and may be older than 0.7 Myr if the initial result from a palaeomagnetically reversed sample above the occupation

floors is confirmed by the scores of other samples now being analysed (Fig. 3) (A. Cox and L. Lee, unpublished data). Details of the K-Ar and palaeomagnetic dating techniques will be given elsewhere.

Pliocene Lake Gadeb came into being as a result of one or more lava flows which dammed back the east-flowing drainage of the ancestral Webi (Figs 1 and 2). The issue of hominid occupation of the Gadeb upland plains is related to the problem of when and why Lake Gadeb finally dried out, which is in turn associated with major tectonic and climatic events in Ethiopia. In this article we interpret the regional stratigraphy and outline the environmental changes at Gadeb during the past 3 Myr. Later papers will deal in detail with the geochronology, prehistory, palaeo-ecology and sedimentology, and are part of a

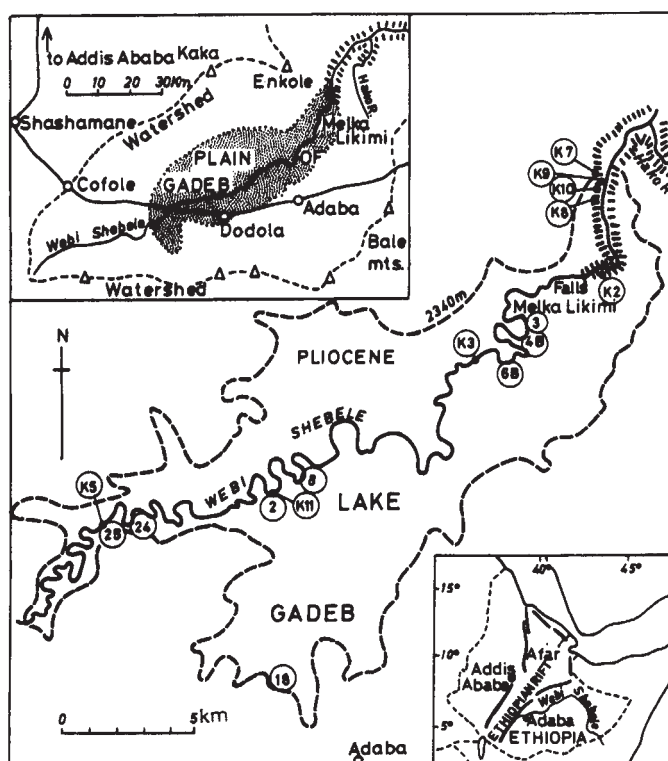


Fig. 1 Location of Pliocene Lake Gadeb, of selected stratigraphic sections (2, 3, and so on), and of K-Ar sample sites (K2 and so on). Triangles along watershed in top left inset map are major volcanoes, often glaciated. Deduced extent of former lake conservative.

(Fig. 4). Analysis of 215 diatomite samples reveals a rich flora (345 taxa) with very diverse assemblages (Fig. 4). The abundance of extinct species makes a detailed ecological interpretation difficult, but it is possible to deduce the ecological influence of certain factors. The relative proportions of planktonic and littoral species allow us to distinguish fluctuations in lake level. On this basis, four major stages, separated by minor regressions, may be recognised (stages I to IV, Fig. 4). Each stage is defined by a set of characteristic species. Despite slight increases in salinity during regressions, the lake was always oligohaline. By comparison with the lakes of East Africa¹⁵, Lake Gadeb was always of low to moderate alkalinity (2–10 mequiv. l⁻¹). Deposition of airfall or reworked ash was also influential, because during stages I and II there is a noticeable peak in the abundance of the ecologically little-known *Stephanodiscus minutus* and *S. tenuissima* immediately above each ash band (Fig. 4). Previous workers have noted that *Melosira* spp. require abundant dissolved silica^{15,16}, and that an influx of volcanic ash can trigger their sudden proliferation¹⁷.

The increase in detrital material towards the top of the lake sequence reflects a fall in lake level, also evident in the diatom flora, followed by progressive drying out of the lake during stage IV. Nevertheless, the silts and sands that accumulated during the final regression of Pliocene Lake Gadeb are sometimes interrupted by thin diatomaceous horizons laid down during a brief return to more swampy conditions. These discontinuous lenses cannot be correlated from one section to the next, and only occur in a few sections near the centre of the basin. We conclude that these deposits do not point to a renewed transgression of the original lake, but in fact correspond to its fragmentation into a series of small swamps, the position of which varied with the changing locus of drainage. These ponds persisted during the ensuing phases of river erosion and deposition contemporary with the arrival of tool-bearing hominids.

Arrival of tool-bearing hominids

As the level of Lake Gadeb fell, alluvial gravels and sands were distributed across the plain by rivers flowing down from the adjacent uplands. Abundant stone flakes, chopping tools, bifaces and cleavers, found in association with broken animal bones, indicate that tool-making groups were occupying the central parts of the plain at this time⁹. The absence of any stone tools or waste flakes in alluvium beneath and immediately above the intact lake deposits at section 18 (Fig. 5), suggests that there were few, if any, tool-making hominids here at this time. Concentration of slightly abraded implements on the floors of shallow channels formed during the final stages of the dwindling lake (Fig. 3) suggests that the hominids probably camped alongside these channels, hunting the animals that lived in, or came down to drink at, the shallow lake remnants near the centre of the basin.

Plio–Pleistocene river deposits

The fluvial sediments that overlie the diatomites are highly variable in particle size and composition, ranging from fine pumice silts to feldspathic sands to basalt gravels. Nevertheless, after allowing for facies changes, a progressive change is evident in the depositional pattern with time.

At localities 2 and 8, the youngest diatomites are capped by an indurated and very distinctive layer of horizontally bedded tuffaceous mudstones (the 'bluff laminites' of Fig. 3). Unconsolidated ashes and clays sometimes underlie the mudstone band, which is in turn overlain by a horizontal bed of coarse basalt gravel (the 'bluff gravels' of Fig. 3), often iron-cemented and containing bone chips and stone tools at locality 2 (excavation 2A, Fig. 3). These tools are the oldest discovered at Gadeb so far, and are separated from younger tool-bearing gravels by a sharp erosional break at the base of bed h (8E Fig. 3).

Above the unconformity at locality 8 are tool-bearing basaltic gravels and feldspathic sands which are capped by a palaeosol on which was found a hippo butchery site (8F Fig. 3), well preserved

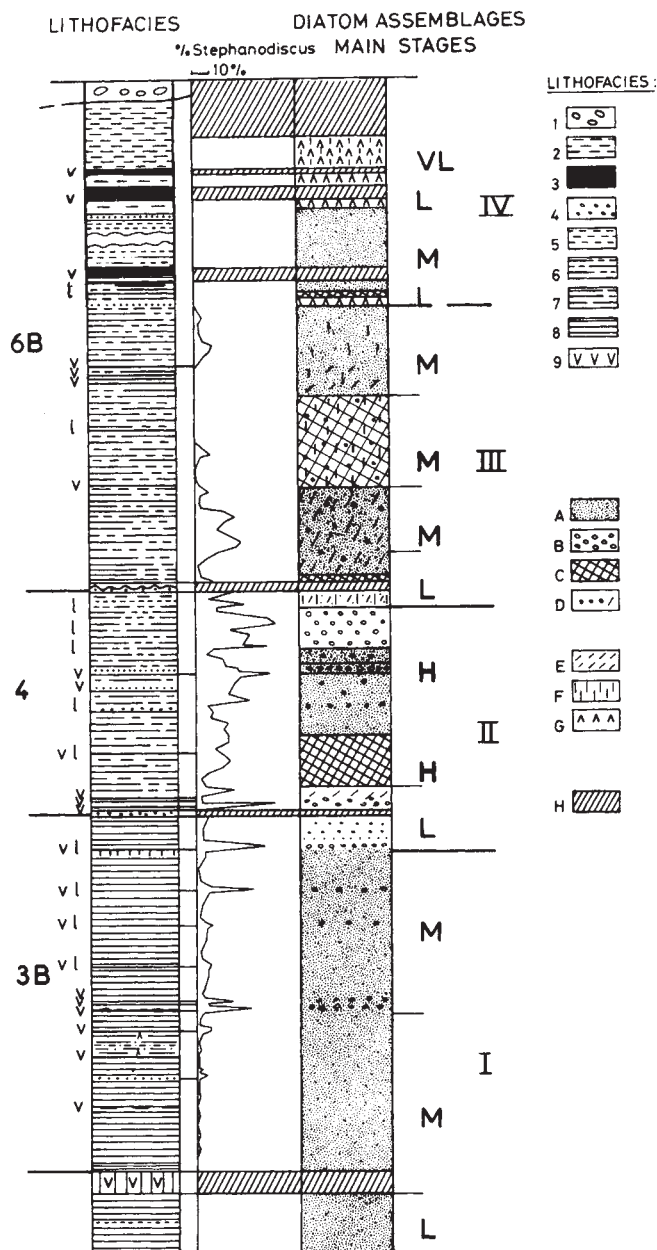


Fig. 4 Pliocene lacustrine sequence at Melka Likimi sites 3B, 4 and 6B, Gadeb plain, Ethiopia. Key to lithofacies: 1, gravels; 2, clay; 3, ash; 4, sand (reworked ash, pumice or feldspars); 5, silt; 6, silty diatomite; 7, clayey diatomite; 8, pure diatomite; 9, basalt; v, volcanic ash; l, limonite band. Key to dominant diatom assemblages: planktonic and tychoplanktonic spp.: A, *Fragilaria* spp.; B, *Stephanodiscus* spp.; C, *Melosira* spp.; D, *Cyclotella* spp.; benthic and epiphytic spp.: E, *Synedra* and *Achnanthes* spp.; F, *Navicula* spp.; G, *Nitzschia*, *Cymbella*, *Epithemia*, *Rhopalodia* and *Pinnularia* spp.; H, no diatoms. Lake level: VL, very low; L, low; M, intermediate; H, high.

beneath a thick layer of finely cross-bedded pumice sands. Several weathered and reworked clayey tuffs overlie these sands and are in turn covered by a wedge of finely laminated pumice silts (the 'upper laminites' of Fig. 3) and by a resistant ledge of pumiceous sandstone conglomerate (Fig. 3).

At locality 2 we also have the sandstone caprock and the eroded bluff gravels. Beneath the upper laminites (m) is a thick channel-fill deposit of loose pumice sand (j) similar to that at locality 8. Unconformably beneath the pumice fill are pumice sands and tool-bearing gravels (Fig. 3, locality 2, 2B, 2C) and a

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Hominid occupation of the East-Central Highlands of Ethiopia in the Plio–Pleistocene

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Stone artefacts and fossil evidence suggest that hominids did not reach the high plateau of Ethiopia until ~1.5 Myr ago. Contact between hominids of the plateau and the Rift Valley seems likely.

THE archaeological localities described here, excavated during the winters of 1975 and 1977, are situated in the Plain of Gadeb on the South-East Plateau of Ethiopia at elevations between 2,300 and 2,400 m in the upper reaches of the Webi Shebele River (see Fig. 1 of ref. 1). Except where human interference has disrupted it, the present vegetation pattern of short, montane grassland with stands of *Acacia* and occasional relict junipers and, in the mountains, *Podocarpus* and juniper forests, is not greatly different from that of the Plio–Pleistocene. The long and very complete sedimentary sequence and its palaeoclimatic and palaeoecological interpretation is summarised by Williams *et al.* in the accompanying article¹.

No stone artefacts or other signs of hominid activity have been found in the earlier part of the succession and cultural evidence appears only when the Pliocene Lake Gadeb had mostly dried out, following the breaching and down-cutting of the dam and the formation of the gorge, at a date between ~1.5 and >0.7 Myr on K/Ar and palaeomagnetic reversal evidence (G. H. Curtis and A. Cox, personal communication). The artefacts occur on palaeosols adjacent to the banks and in association with the gravels of shallow stream courses draining into the ponds and swamps that still occupied the lower and central parts of the basin. The cultural horizons are exposed by the meanders of the main river and so occur in the more central parts of the exposed floor of the former Pliocene lake. Examination of the southern flanks near the base of the Bale Mountains (which rise to over 4,000 m) produced only occasional artefacts of Developed Oldowan type suggesting that the more outlying parts of the basin may have been less favourable living places for hominids.

The evidence from Gadeb suggests that hominids appeared on the Ethiopian high plateau only after ~1.5 Myr perhaps

~1.0 Myr ago, at a time when *Homo erectus*, the maker of the Acheulian and Developed Oldowan industrial complexes^{2,3}, was moving out of the drier and warmer, though well-watered, more low-lying traditional habitats in the Rift Valley and on adjacent parts of the African plateau and was beginning to colonise most ecological niches in Africa—including the high plains.

The Pliocene sediments exposed in the central parts of the basin appear to have been mostly laid down in standing water and so are unlikely to have artefacts associated with them. Only towards the western end, at geological sections 24 and 25¹, is a palaeosol preserved beneath a capping of welded tuff dated to 2.35 Myr (G. H. Curtis personal communication). This palaeosol has yielded part of an elephant molar, perhaps an early form of *Elephas recki*, but the horizon must be examined before we can be certain that no artefacts or other evidence of hominid activity are associated with it.

Developed Oldowan occurrences

The main artefact localities are shown in the accompanying article¹, the most important being localities 2, 8 and 25 and those that line the eastern shoulder of the Webi Gorge downstream from the Falls to the Hako confluence. With the possible exception of the Acheulian artefacts from the gravels above the Gorge, which cannot, as yet, be tied into the stratigraphic sequence upstream, the oldest assemblages recovered occur at locality 2 and come from the iron-stained base of the ferruginous gravels (f) that rest on the mudstones (e) which, in turn, overlie disconformably the Pliocene diatomite. A small excavation, 2A, showed that artefacts and bone fragments occurred in a relatively limited area of 6 × 2.5 m and, as they are mostly moderately abraded, were not in primary context. The artefacts comprise a handaxe, choppers, core-scrapers, polyhedrons, irregular hard-hammer flakes and the cores from which they were struck, together with a few regularly retouched tools, all from basalt cobbles. A selection is shown in Fig. 1. The assemblage is provisionally classified as Developed Oldowan but the retouched scrapers show a refinement more usual with the Acheulian tradition. Although similar occurrences of choppers,

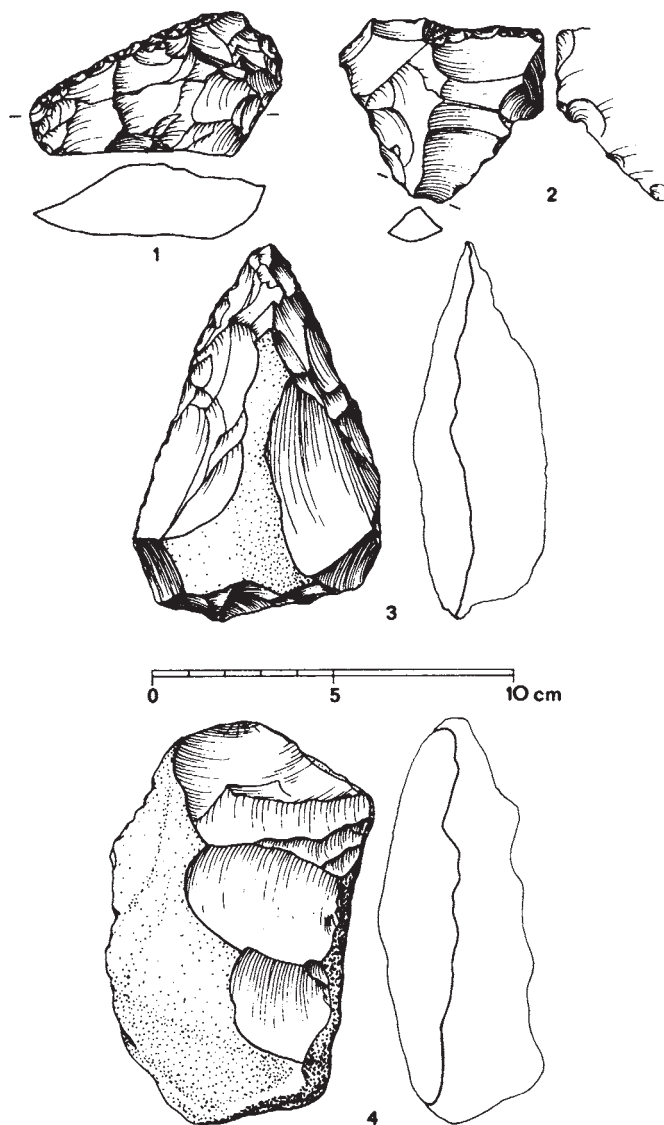


Fig. 1 Artefacts of Developed Oldowan B. tradition from Excavation 2A at Gadeb Locality 2. 1, Side scraper; 2, modified and retouched side-flake; 3, sub-triangular handaxe; 4, side chopper, modified. Material—basalt.

heavy and light duty scrapers and polyhedrons are known from other late Lower and Middle Pleistocene localities in Africa, these are, perhaps, the first such assemblages to have been assigned to the Developed Oldowan B outside the type locality at Olduvai Gorge.

The next oldest Occurrence excavated is found several hundred metres east of 2A, in a channel fill cut into a pumice mudflow dated to 1.48 Myr (G. H. Curtis, personal communication). This channel is 30–40 m wide and filled with alternating deposits of silt, fine sand and clays with occasional thin, discontinuous layers of basalt cobbles, rounded angular fragments of ignimbrite, artefacts and bone in a matrix of sand and fine gravel. An excavation (2E) of 40 m² was made in one such horizon towards the eastern edge of the channel and uncovered a hominid activity area, apparently occupied at a time when the channel floor was temporarily exposed. The horizon dips gently from east to west towards the centre of the channel where the gravel is ~10 cm thick but, towards the bank the artefacts and cobbles rest directly on the underlying clayey silt. The silts and clays overlying this horizon contain many pollens and diatoms and the extent to which this Occurrence has been reworked by fluvial action can be assessed by the fact that the

diatoms still preserve their fragile spiny structure which would undoubtedly have been broken had there been any turbulence in the water that laid down these beds. Most of the artefacts are slightly (basalt) to moderately (welded tuff) abraded, suggesting that 2E represents a primary context site which has suffered some but no extensive resorting before being fairly rapidly covered by renewed depositional activity.

The assemblage, 1741 specimens, is typical of the Developed Oldowan B Complex (pages 258–281 of ref. 2), comprising the usual range of choppers, core-scrapers, polyhedrons and sub-spheroids, flake scrapers and modified pieces together with cores, flakes and flaking waste (Fig. 2). There are also six handaxes and one cleaver, all exhibiting hard-hammer technique and showing a lack of the refinement normally found with the Acheulian assemblages at Gadeb. Note that unmodified cobbles comprise only 18.4% and simply split cobbles 4.2% of the total. If the assemblage were all fluvially deposited one would have expected many more cobbles than artefacts which suggests that the former may have been carried onto the site by the hominids to use for making tools.

A number of fossil bone fragments and teeth, ranging from fresh to moderately abraded, are associated with the artefacts. Bones, mostly broken into small pieces, and teeth came from hippopotamus, elephant, various bovids, zebra and pig. No concentrations could be discovered but the assemblage is an indication of the game animals available on the high plateau at this time (faunal identifications at Gadeb by D. Geraards and S. Edwards are provisional).

Higher in the 22-m sequence at Locality 2, are stratified basalt gravels with artefacts, overlying weathered tuffs. Two small excavations made in 1975 exposed parts of two further concentrations of Developed Oldowan B character, both in channel fills with, in each case, no significant resorting by subsequent stream action. The earlier occurrence (2C) rested on the weathered tuffs and was covered by fine, current-bedded sands ~1 m thick. A total of 622 artefacts, with some small, unidentifiable bone fragments, came from a 3.5 m × 1.5 m excavation and comprise 12.5% shaped and modified pieces and 87.4% unmodified waste: 66 are shaped tools—choppers, polyhedrons, 5 rather poorly-made handaxes, 4 other bifacially worked artefacts and 27 small scrapers on flakes, flake fragments and chunks, the commonest form being a single-side scraper. There are also 12 pieces with modified edge damage. The artefacts showed no discernable orientation or concentration and were fresh to moderately abraded.

The 2B site, a little higher in the basalt gravels, yielded 589 artefacts from a 2 × 4 m excavation, shaped tools representing 9.3%, modified 0.3% and unmodified waste 90.3%. Again the assemblage can be classified as Developed Oldowan B with the usual choppers, polyhedrons and scrapers. There are also one proto-biface, one handaxe and one cleaver, again poorly-made in comparison with those from the Acheulian. When compared, all three of these Locality 2 assemblages (2B, 2C and 2E) are seen to be similar. In addition, comparison with assemblages of this Complex from East Africa confirms that the Gadeb occurrences belong with the Developed Oldowan B, or perhaps C (from Beds III and IV at Olduvai Gorge), although this has, as yet, not been described in detail^{4–6}.

Acheulian occurrences

The earliest Acheulian assemblages yet found at Gadeb occur at Locality 8 associated with basalt gravels and felspathic sands resting on an erosional unconformity cut down into the later Pliocene diatomites. This horizon has been excavated in three places—two (8A and 8D) in 1975 and the third (8E) in 1977¹. The first two were moderately small but that at 8E exposed ~100 m² of the occupation horizon (Fig. 3). Again, the context is a shallow stream channel cut across the floor of the Pliocene Lake Gadeb and draining into the central lakelets and swamp. The small 8A excavation produced the surprising number of 1,849 artefacts of which 251 were handaxes and cleavers, most

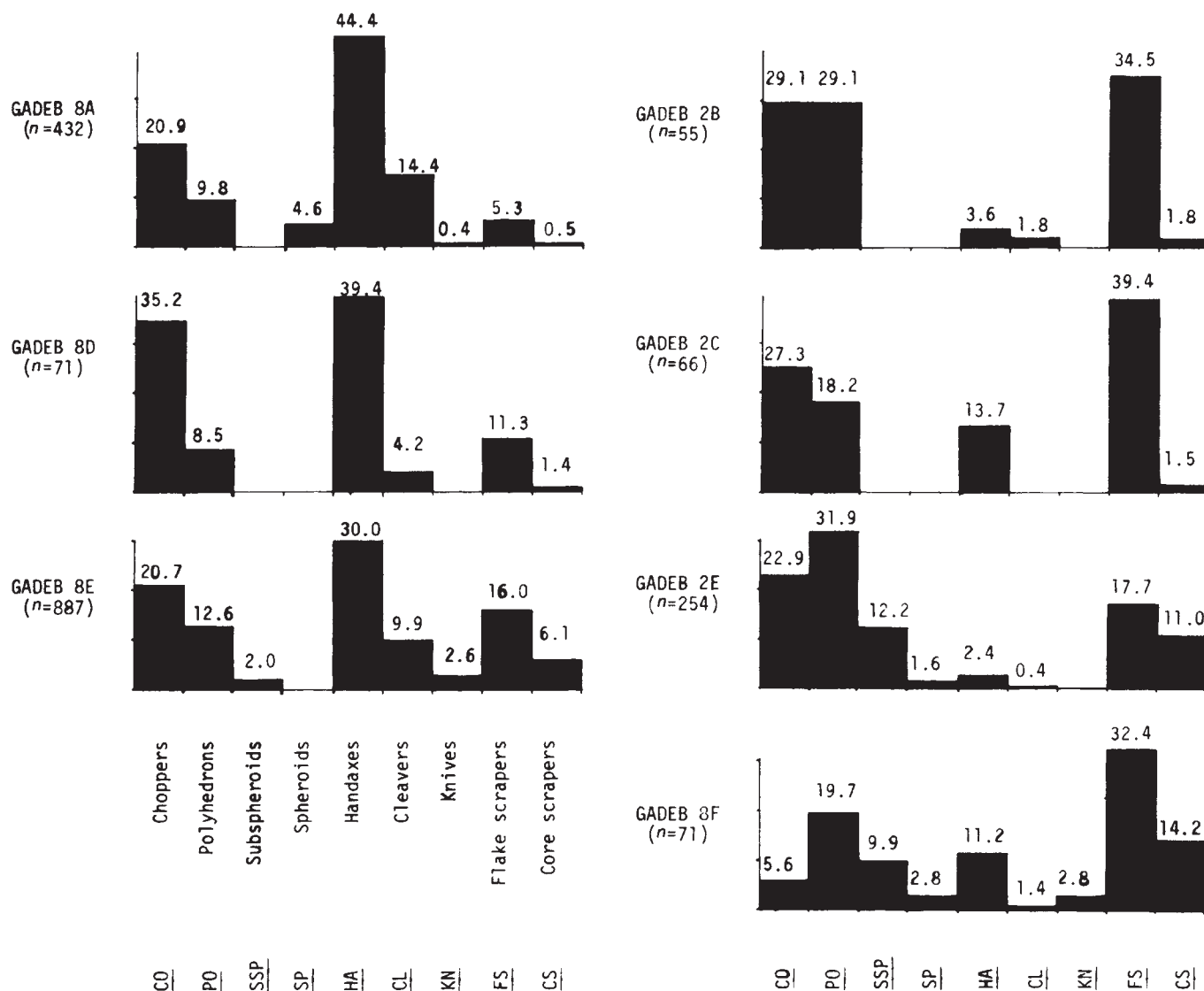


Fig. 2 Histograms comparing Acheulian (left) and Developed Oldowan (right) tool-kits from excavated sites at Gadeb, east central Ethiopia. Each division on the vertical axis represents 10 per cent.

moderately to heavily abraded and their alignment and imbrication show clearly that the assemblage had been incorporated in the stream gravels. Excavations 8D and 8E were ~30 m apart and exposed the edges of a channel and its banks to the south-east. To the south and west the gravel was 10–15 cm thick while at the eastern end the artefacts rested directly on the diatomite. The 8D excavation (3×5 m) revealed a primary context concentration of artefacts and fossil bone, including two complete hippopotamus canines and fragmentary remains of bovids, zebra and elephant as well as unidentifiable bone fragments. The 487 artefacts were mostly fresh to slightly abraded and 14.6% were shaped tools, 1.6% modified and 83.8% unmodified waste. Bifaces comprise 43.6% of the shaped tools—mostly handaxes with three cleavers—the remainder being flake scrapers, choppers and spheroids indistinguishable from similar tools associated with the Developed Oldowan.

The 8E excavation in the same occupation surface yielded 20,276 artefacts and a small quantity of fauna. Except for a nearly complete zebra mandible, the bone consisted mostly of unidentifiable small fragments and isolated teeth, the commonest of these being hippo represented by two complete, large incisors and several molars and molar fragments. The next commonest group were the bovids followed by zebra. Again, there were no bone accumulations and it would appear that

butchery was not a major activity here or at the other Acheulian sites excavated at Gadeb.

The greater number of artefacts occurred in the northern half of the excavation due, probably, to gravitation down-slope. No preferred orientation is apparent and the bifaces lay flat on the surface (Fig. 3). 45% of artefacts are moderately, and 26% slightly abraded; 15% are fresh. It seems that the assemblage has suffered slight movement, though insufficient to disperse or significantly alter the concentration before it was buried under felspathic sands with silt and clay lenses in the lower part.

The composition of the 8E assemblage is given in Fig. 2: 4.4% of the pieces are shaped tools, 10.5% are modified or show edge damage and 50% is unmodified waste the remaining 35% being cobbles and split cobbles. The commonest shaped tools are bifaces: handaxes (25%), cleavers (9.9%), knives (2.6%) and other (or broken) bifaces (4.9%). The next most numerous are flake scrapers (16.0%) among them several continuously retouched and convergent forms. The other tools—choppers, polyhedrals, spheroids and core scrapers—are similar to those found with the Developed Oldowan (Fig. 4).

The raw material is basalt and ignimbrite with a little chert and obsidian. The ignimbrite was obtained from outcrops, the nearest today being several kilometres to the west and south. The ignimbrite handaxes were made from large flakes struck

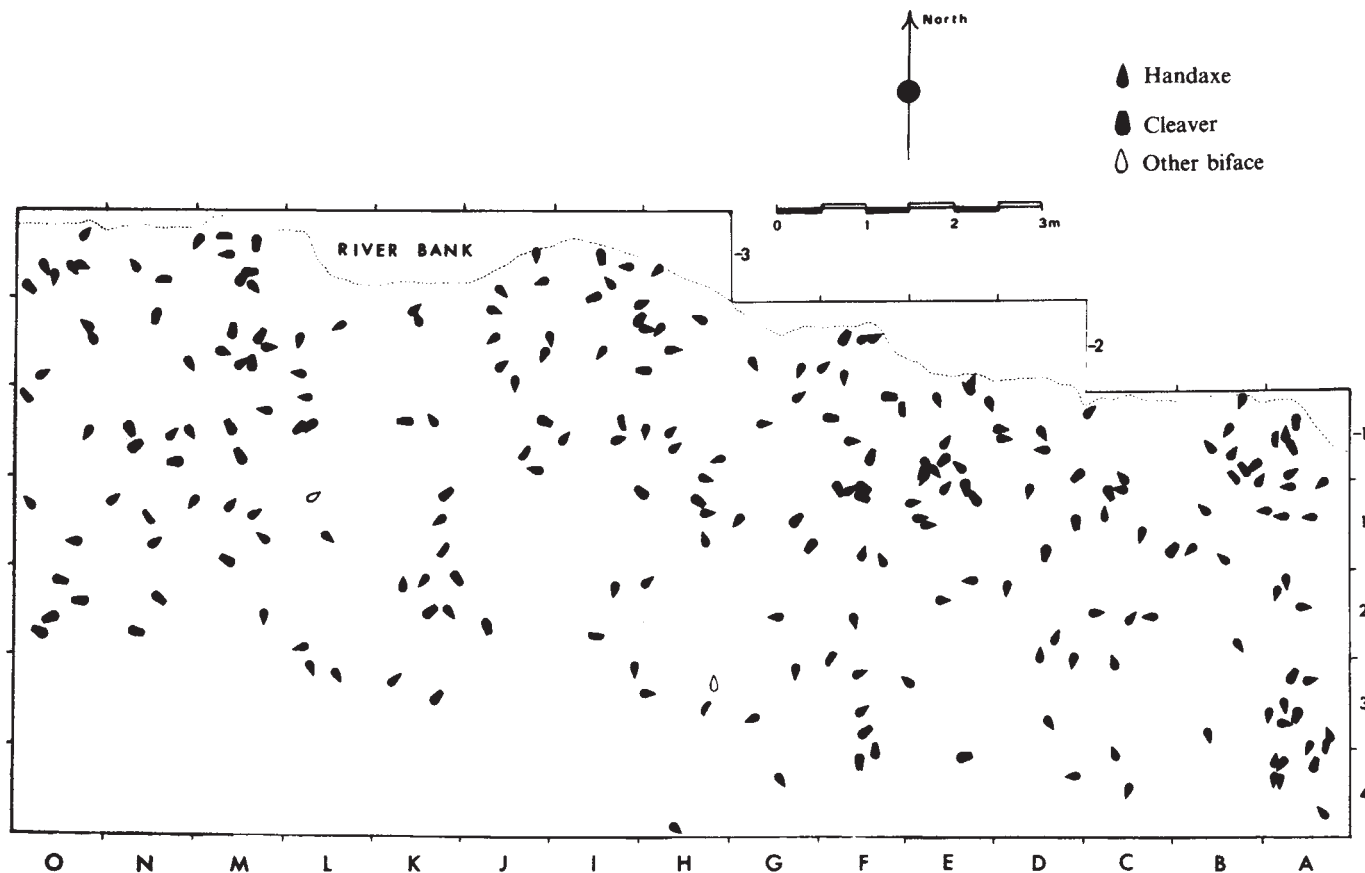


Fig. 3 Plot of the distribution and orientation of the bifaces (handaxes, cleavers) on the Acheulian occupation horizon at Gadeb 8E, 1977.

from these outcrops and are larger, broader, thicker and more elongated than those made from basalt cobbles which are generally ovate or long ovate in plan form (Fig. 4, no. 9). The large flakes from cobbles, retouched as handaxes and cleavers, usually have a flat release face which often results from splitting a cobble by throwing (N. Toth, personal communication). Bifacial handaxes are commonest but an interesting form occurs flaked only on the release face and exhibiting inverse retouch; occasionally, these are also minimally retouched on the cortex face. Flake scrapers also sometimes show this form of retouch. (Biface plan forms in relation to raw material at 8E are shown at Fig. 5).

Four ovate handaxes in obsidian from 8E are of special interest since they—or the raw material—must have come from a considerable distance. No sources of obsidian are known on the South-east Plateau the nearest being to the west in the Rift or on the escarpment edge ~100 km away¹. This distance is appreciably greater than any hitherto known for the Lower Palaeolithic and has important implications, therefore, for movement or group contacts between the Plateau and the Rift.

Among the modified or utilised artefacts are 11 cobbles, or rounded fragments, with a pit formed in the centre of one face by hammering with some pointed implement (Fig. 4). Similar pitted stones have been found at Olduvai Gorge (upper Bed II and Beds III/IV)⁷, and on the Developed Oldowan horizon Gomboré I at Melka Kunture on the Ethiopian Plateau⁸. They are believed to be anvils used with hammer stones in the bipolar flaking method although other uses cannot be excluded.

The occupation floor also yielded several fragments of heavily weathered basalt which, when rubbed, give a red pigment. None of the pieces show unquestionable evidence of rubbing but the possibility should not be ignored. Several pieces of welded tuff showing what appears to be alteration by fire were also recovered, including several together in a concentration. Palaeo-

magnetic measurements by Dr M. Barbetti, University of Adelaide, on samples from the 8E activity area have produced suggestive, but not conclusive evidence for the use of fire by Acheulian man at Gadeb.

Butchery site

On the surface of the palaeosol overlying the felspathic sands covering the Acheulian occupation at Locality 8 (Fig. 3, ref. 1), was found, at Excavation 8F, a discrete concentration of artefacts associated with part of a butchered hippo (Fig. 6). The 385 fresh or slightly abraded artefacts are presumed to be in primary context; 18.4% are shaped tools, 7.8% are modified or show edge damage and 3% are miscellaneous pieces (Fig. 2). Only 51.2% represent unmodified waste and 19.2% are cobbles or split cobbles all of which must have been carried onto the site. The commonest tools (32.4%) are small flake side-scrapers; polyhedrons and spheroids also account for 32.4% and there are 14.1% of core-scrapers. Choppers are few (5.7%) and bifaces are represented by a cleaver, three small, poorly-made handaxes, two knives, similarly of crude workmanship and five miscellaneous forms. Among the modified/utilised artefacts are 17 flakes and flake fragments. This tool-kit could have been used in butchering the hippo and the polyhedrons, spheroids and some of the small tools were found in close association with the bones.

Technically this assemblage belongs and is assigned to the Developed Oldowan B complex. The palaeosol is overlain by silts with reversed magnetism and has an age of ≥ 0.7 Myr.

Quarry site

An important Acheulian ignimbrite quarry and workshop was discovered at the end of the 1977 season at Locality 25, ~6.5 km west of locality 2 where basalt gravels, overlain by ash and

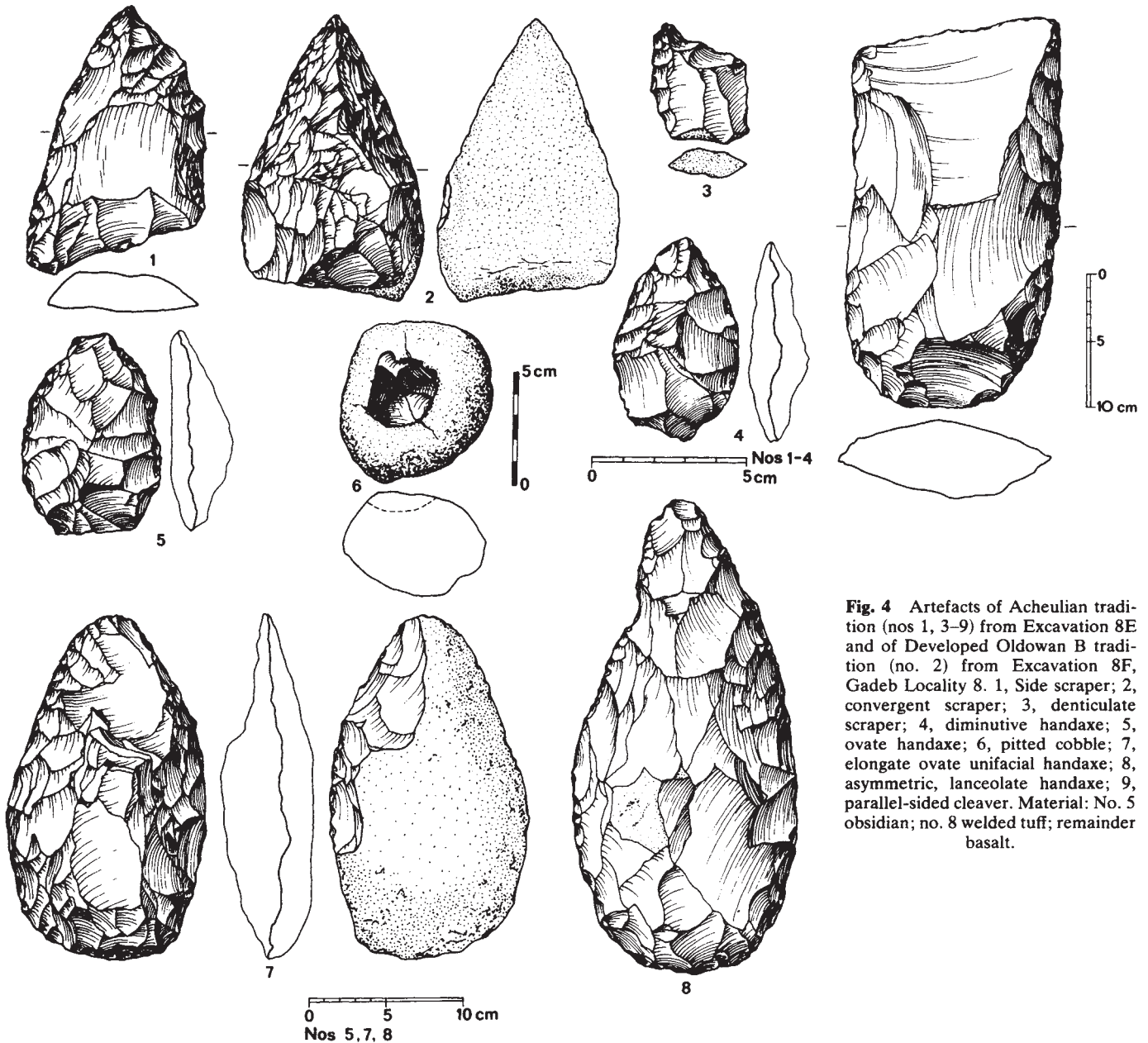


Fig. 4 Artefacts of Acheulian tradition (nos 1, 3-9) from Excavation 8E and of Developed Oldowan B tradition (no. 2) from Excavation 8F, Gadeb Locality 8. 1, Side scraper; 2, convergent scraper; 3, denticulate scraper; 4, diminutive handaxe; 5, ovate handaxe; 6, pitted cobble; 7, elongate ovate unifacial handaxe; 8, asymmetric, lanceolate handaxe; 9, parallel-sided cleaver. Material: No. 5 obsidian; no. 8 welded tuff; remainder basalt.

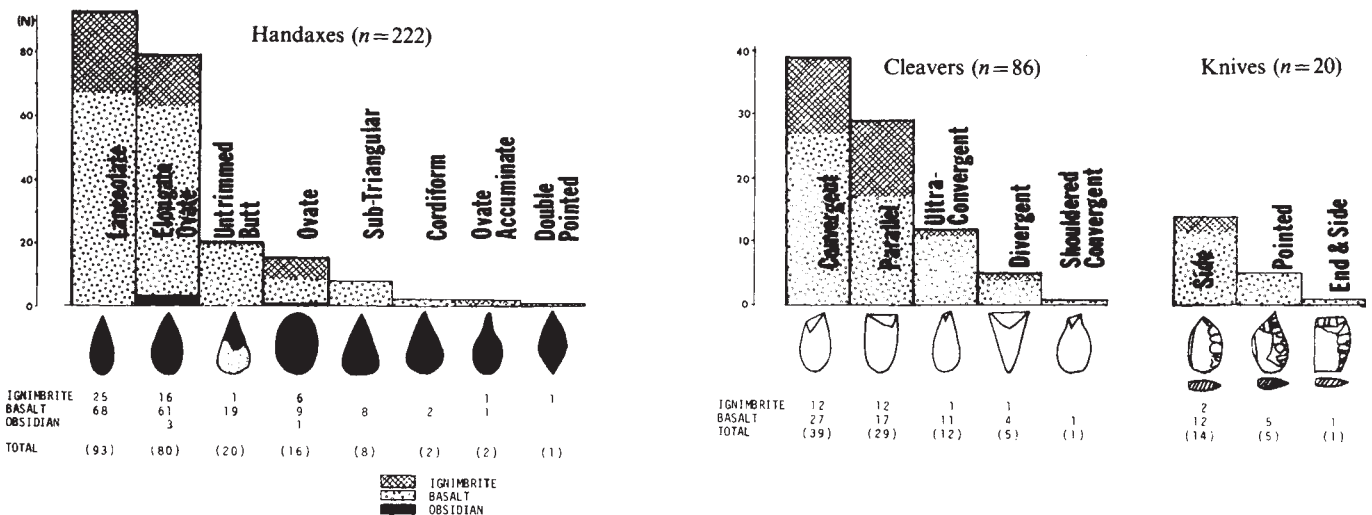


Fig. 5 Acheulian biface forms and raw materials at Gadeb 8E, 1977.

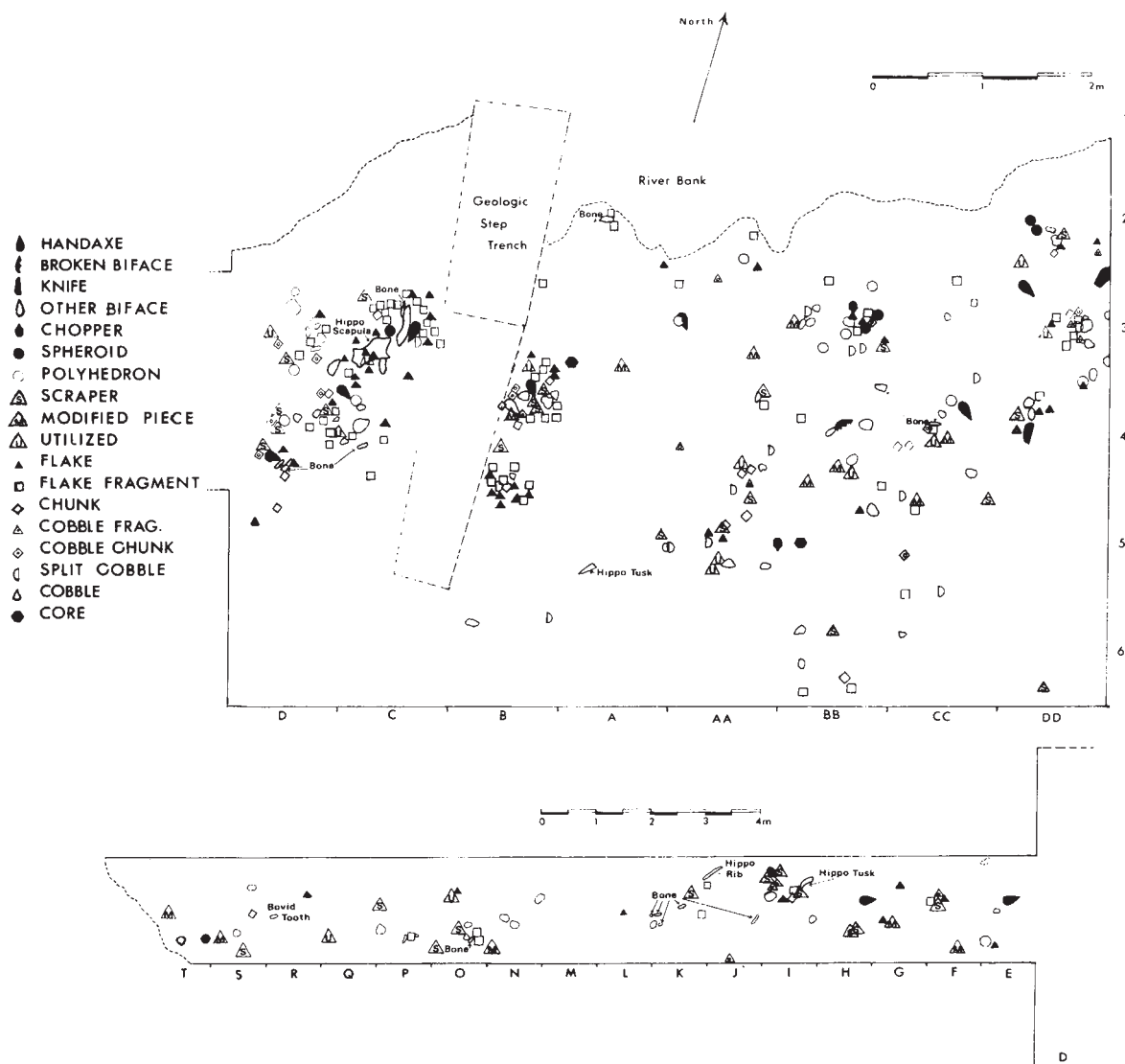


Fig. 6 Plan of the hippo butchery concentration on the palaeosol at Gadeb 8F, 1977.

pumice sandstone, fill an erosion channel cut into the earlier Pliocene sediments capped by welded tuff (Fig. 5 of ref. 1). A small trial trench yielded 112 Acheulian artefacts in fresh condition together with fossil bone. The ignimbrite had been 'mined' by knocking off large flakes from the outcrop and fallen blocks and there is no evidence of the proto-Levallois technique. This is one of the few sealed Acheulian quarry sites known to us but, without further work, it cannot be correlated directly with the succession at localities 2 and 8.

Either contemporary with or somewhat later than the 8F butchery site are two well-made, fresh Acheulian cleavers with some fossil bone which had come from the base of the bedded pumice sands (j) in the deep channel fill beneath the 'upper laminites' (m) at locality 2 (Fig. 3 of ref. 1). These are the youngest of the Lower Pleistocene Occurrences at Gadeb, as no tools have, as yet, been found in the Pumice Sandstone that caps the succession.

In the gravels at the top of the Webi Gorge between the Falls and the Hako confluence, concentrated patches of Acheulian bifaces and other tools occur in secondary context. At the Hako confluence itself, residual gravel patches yielded weathered, but not especially water abraded, choppers, cores and flakes of Developed Oldowan form. Clearly, therefore, Acheulian man was present during the final breaching of the dam and while the river was cutting back upstream as far as the lower of the two existing waterfalls.

Conclusions

It seems probable that hominids did not penetrate to the high plateau until after the Acheulian complex appeared in the Rift after 1.5 Myr ago. The Gadeb sites show an alternating sequence of Acheulian and Developed Oldowan occurrences dating between ~1.5 and >0.7 Myr. These must have been contemporary traditions the main distinguishing feature being the bifaces with the Acheulian, whose makers were, therefore, engaged in biface-related activities not common with the Developed Oldowan. The bone waste found on the sites, however, indicates that butchery was more commonly practised by the makers of the Developed Oldowan using the chopper, polyhedron and small tool element. The sites were generally close to or in stream courses, as elsewhere in East Africa and Ethiopia^{9,10} and, while remains of grazing animals are found, the preferred food animal appears to have been the hippo. The Gadeb assemblages show generally close similarities, though with local variation, with those from sub-Saharan Africa as a whole.

The hominids seem to have been relatively far ranging and contact between plateau and Rift seems likely. *Homo erectus*, even at this unusually early time, may have been experimenting with pigment at Gadeb and also with fire; the latter, perhaps, induced by the cold temperature of the plateau since this must be one of the highest altitudes at which assemblages of this time range have been found in either Africa or Eurasia.

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Isolation and characterisation of a yeast chromosomal replicator

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A yeast DNA sequence that behaves as a chromosomal replicator, ars1 (autonomously replicating sequence), has been isolated. On transformation, ars1 allows autonomous replication of all co-linear DNA. The replicator can integrate into other replication units and can function in multimeric form. The 850-base pair ars1 element has no detectable homology to other yeast sequences. Such replicator-containing plasmids can be used for the isolation of DNA sequences in yeast cells as well as for the study of chromosomal DNA replication.

THE replication of a cell's genome must be properly controlled to harmonise with the many other functions required for cell division. In bacteria, the rate of DNA replication is controlled at the onset of synthesis^{1,2}. The initiation event occurs at a single site on the bacterial chromosome³. This initiation site or origin of replication has been genetically defined as the 'replicator' (ref. 4), an element that allows replication of all DNA attached to it. Several prokaryotic replicators have been isolated (see ref. 5 for examples) and their further biochemical and genetic analysis will help elucidate the molecular mechanism(s) that control the initiation of bacterial DNA synthesis.

How is DNA replication controlled in the larger genomes of eukaryotes? An abundance of physical data indicate that eukaryotic chromosomes are organised into tandem replication units (reviewed in refs 6–8). The rate of DNA chain elongation seems to be the same for all the replication units in a cell, whereas different units initiate DNA synthesis at different times. Furthermore, initiation is a non-random event. Replication units begin synthesis in a defined order and individual units demonstrate the same temporal specificity in successive divisions. However, the paucity of genetic and biochemical evidence means that it is impossible to determine whether such replication units are strictly analogous to prokaryotic chromosomes. For example, it has been proposed that initiation occurs not at a specific DNA sequence, but at a random site, determined solely by the spatial arrangement of the DNA within the highly structured chromosome^{6,7}.

During our studies on transformation of the yeast, *Saccharomyces cerevisiae*, we identified a segment of

chromosomal DNA that could replicate without integrating into the yeast genome⁹. Here we further characterise this autonomously replicating segment. Its behaviour suggests that the DNA sequence contains a eukaryotic chromosomal replicator.

ars1: A chromosomal replicator

A 1.4-kilobase pair *Eco*RI endonuclease-generated DNA fragment, containing the centromere-linked *trp1* gene was isolated (D.T.S. and R. W. Davis, in preparation). This fragment (Sc4101) was inserted into the *Eco*RI cleavage site of the bacterial plasmid, pBR322 (ref. 29) (Fig. 1). The resulting plasmid is termed YRp7. Covalently closed, supercoiled YRp7 DNA was used to transform a *trp1*[−] yeast strain. The efficiency with which these cells were transformed to Trp⁺ was approximately 1,000-fold higher than the efficiency observed with other segments of yeast chromosomal DNA⁹. Furthermore, the high frequency of transformation was associated with autonomous replication. When DNA was isolated from these transformants and analysed by restriction endonuclease digestion and DNA–DNA hybridisation, the transforming sequences were detected as supercoiled, circular DNA and were not found integrated into the host genome. Presumably, the higher frequencies of transformation arise because autonomous replication allows heritable expression without requiring chromosomal integration via a less frequent recombination event. Thus, high transformation efficiency can be used as an indicator for autonomous replication of these hybrid molecules.

To test whether autonomous replication might be due to the particular sequences at the yeast/pBR322 junctions, the transformation efficiency of YRp7', which contains the Sc4101 insert inverted relative to YRp7, was measured. Both YRp7 and YRp7' gave rise to 2,000–4,000 Trp⁺ colonies per µg DNA. As other yeast sequences (for example the *his3*, *leu2*, *ura3* structural genes^{9,11,12}) fail to replicate autonomously when inserted into pBR322, it is likely that the element that permits autonomous replication is located on Sc4101 itself. This was further investigated by inserting the Sc4101 DNA fragment into YIp1, a *his3*-containing yeast vector that transforms at low efficiency via integration into the host chromosome (Fig. 1). As expected, the frequency at which YRp7–Sc2605 and YRp7'–Sc2605 DNAs transformed *his3*[−] to His⁺ was also high (2,000 His⁺ colonies per µg DNA). Sc4101 has been joined to many

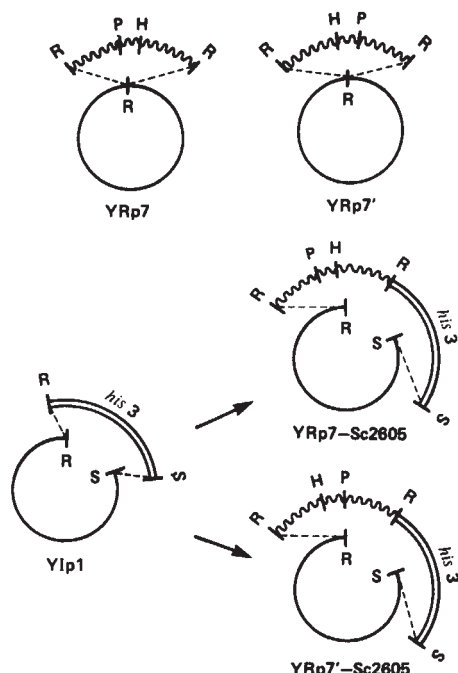


Fig. 1 Structure of several yeast replicator plasmids. Solid lines depict pBR322 plasmid sequences, wavy lines represent the *trp1*-containing 1.4-kilobase pair yeast fragment (Sc4101) and open bars a 6.0-kilobase pair yeast fragment (Sc2605) that contains the *his3* structural gene. Not to scale. YRp7 and YRp7' were constructed by the ligation of an *Eco*RI digest of λ gt-Sc4104 and pBR322 as described previously⁹. YRp7-Sc2605 and YRp7'-Sc2605 were similarly constructed by insertion of the Sc4101 fragment in either orientation into the *Eco*RI cleavage site of YIp1. *Eco*RI was the gift of Marjorie Thomas. Other restriction endonucleases and T4 DNA ligase were from New England Biolabs or Bethesda Research Laboratories and were used as directed. After ligation, the hybrid molecules were transformed³¹ into *trpC9830*, an *E. coli* strain lacking *N*-(5'-phosphoribosyl)anthranilate isomerase. The presence of Sc4101 was then determined by testing for complementation of the bacterial lesion by the yeast *trp1* gene (D.T.S. and R. W. Davis in preparation). The orientation and fidelity of the purified plasmid DNA were assessed by restriction endonuclease digestion and horizontal agarose gel electrophoresis¹⁰. Transformation of D13-1A (*a trp1 his3-532 gal2 SUC2 mal-*) was carried out as described previously⁹. The transformation efficiencies for the YR plasmids cited in the text were determined on several separate occasions using other yeast vectors to control for experimental variation.

other yeast and bacterial sequences, producing hybrid molecules 4 to 50 kilobase pairs in total length. All these Sc4101-containing DNAs exhibited high frequency transformation and, concomitantly, replication in the absence of recombination with the host genome (S. Scherer, T. P. St. John, D.T.S. and K.S., unpublished observations). These data are consistent with the idea that Sc4101 contains, in addition to the structural sequence for the *trp1* gene, a genetic element that allows autonomous replication, which we will call *ars1* (for autonomously replicating sequence).

Yeast cells can be transformed simultaneously by two different DNA species at high frequency¹³. For example, when a mixture of YRp7-Sc2605 and YRp12 (YRp7' with a 1 kilobase pair yeast DNA fragment carrying the *ura3* gene inserted at the *Ava*I endonuclease cleavage site¹²) DNA was added to a *ura3*⁻*his3*⁻ strain, Ura⁺His⁺ co-transformants arose at the same frequency (2,000–5,000 colonies per μ g) as Ura⁺ or His⁺ transformants. So, in these transformation conditions, DNA is saturating and each yeast cell is capable of receiving multiple DNA molecules. This observation can be used to design a *cis/trans* test for *ars1* function. If *ars1* functions in *trans*, co-transformation of a *his3*⁻*trp1*⁻ yeast strain by YIp1 and YRp7 DNAs should lead to a 1,000-fold increase in His⁺ colonies compared with transformation by YIp1 alone. However, the mixture of YIp1 and YRp7 DNA gave rise to only 78 His⁺ colonies per μ g DNA. Concurrently, YRp7-Sc2605 DNA gave rise to 2,000 His⁺ colonies per μ g DNA. Thus Sc4101 allows

high frequency transformation and autonomous replication of the *his3* sequence only when located on the same molecule. As *ars1* is linked to the yeast chromosomal *trp1* locus and behaves as a *cis*-acting effector of DNA replication, we propose that *ars1* is a yeast chromosomal replicator.

Autonomously replicating *ars1* hybrid molecules are unstable

In a minimal medium supplemented with histidine and tryptophan, a *trp1*⁻*his3*⁻ yeast strain will grow with a doubling time of 2.5 h. When transformed by YRp7-Sc2605 to His⁺Trp⁺, the strain doubles in 3.5–4 h in the absence of histidine and/or tryptophan. Similarly increased generation times are observed for all other *ars1*-containing transformants, regardless of the selective marker used. It is thus unlikely that the slow growth rate is due to a deficiency in expression of the transformed genes but is due rather to mitotic loss of the autonomously replicating hybrid DNA. In a logarithmically growing minimal medium culture of a *his3*⁻ strain transformed by YRp7-Sc2605 DNA only 30–50% of the cells are phenotypically His⁺. If this culture is diluted into a non-selective medium, the proportion of His⁺ cells drops to 1–5% within 10 generations. The resulting *his3*⁻ cells do not contain any hybrid DNA as determined by DNA-DNA hybridisation (data not shown).

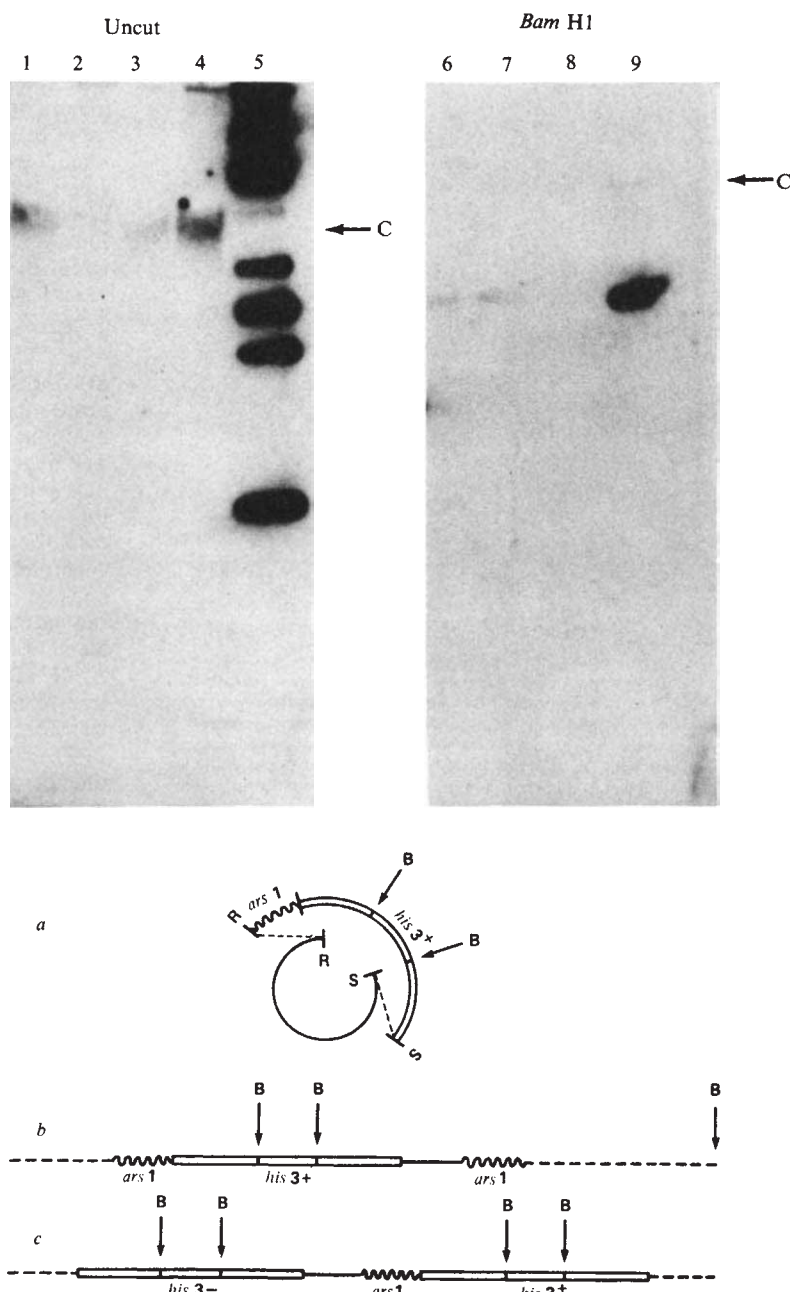
There are several possible explanations for the mitotic instability of *ars1*-containing hybrid molecules. The isolated replicator may not contain all the information necessary for complete replication during each cell cycle. However, larger DNA fragments that contain additional DNA sequences adjacent to *ars1* are still mitotically unstable (D.T.S. and R.W.D., in preparation). Alternatively, *ars1*-containing extra-chromosomal elements may compete with the host chromosome for limited initiator(s). This is one proposed explanation for the instability of plasmids containing the *Escherichia coli* origin of replication^{15,16}. Hybrids containing *ars1* plasmids may replicate during each cell cycle but may fail to segregate properly during mitosis. In the last case, addition of other DNA sequences controlling chromosomal segregation would be expected to stabilise a replicator plasmid.

Integration of *ars1* hybrid molecules

The slow growth of strains containing *ars1* hybrid molecules results in a strong selective pressure favouring any stably transformed cells. Such transformants can indeed be isolated. After many generations of growth in selective or non-selective conditions, cells were plated on non-selective medium. These colonies were tested for the stability of the transformed phenotype by replica plating to a selective medium. Stable transformants isolated in this way grew at the parental rate in minimal medium. The fate of the transforming DNA was investigated in four independently isolated stable strains (Fig. 2). The diagram at the bottom of Fig. 2 shows the digestion patterns expected for three possible conformations of the foreign sequences. The transforming DNA was identified by hybridisation and co-electrophoresed with the high molecular weight linear host DNA in all four cases (Fig. 2, lanes 1–4). The structure of the integrated DNA in one transformant was analysed by digestion with *Bam*HI endonuclease. The foreign pBR322 sequences in the stable transformant were detected as a single band (Fig. 2, lane 7) co-migrating with the *Bam*HI fragment from an unstable transformant (lane 6) and from the original plasmid DNA (lane 9). These data indicate that YRp7-Sc2605 is integrated at the *his3* locus without major sequence alteration.

Given the appropriate genetic markers, stable transformants may be directly selected. Fragments containing non-complementing derivatives of the *his3* gene (the missense mutations *his3-38* and *his3-532* (ref. 17), as well as an amber mutation¹⁸ and two deletions¹⁹) were inserted into YRp7. These hybrid molecules were introduced into a *his3-532trp1*⁻ strain by transformation to Trp⁺. Although the transformants were initially His⁻, His⁺ recombinants could be selected at a frequency of 10⁻⁵–10⁻⁶ per generation. The only exception to this result

Fig. 2 Analysis of stable *ars1* transformants. DNA was rapidly purified from transformants grown in minimal media (6.7 g l^{-1} Difco yeast nitrogen base, 2% glucose) as described previously⁹. In 'uncut', the DNA from four stable transformants described in the text was electrophoresed without endonuclease digestion in a 0.4% agarose gel (lanes 1–4). Lane 5 contains the original transforming DNA. The multiplicity of bands (several of which showed lower mobilities than the yeast chromosomal DNA) is apparently due to multimeric and nicked forms of the plasmid. In 'BamHI', DNA isolated from an unstable transformant (lane 6), a stable, integrated transformant (lane 7), the parental strain (lane 8), and the transforming plasmid DNA (lane 9) were digested with *Bam*HI restriction endonuclease and electrophoresed on a 0.4% agarose gel. The DNA in each gel was transferred to a sheet of nitrocellulose paper²⁵. ³²P-labelled pBR322 DNA was prepared by nick-translation²⁶, heat denatured, and hybridised to the immobilised DNA spectra. These operations were carried out using modifications of the traditional procedures as described in ref. 27. Pictures of the two autoradiographs are presented here. Also depicted are three possible structures of transformed *ars1* sequences: *a*, unintegrated and autonomously replicating; *b*, integrated by homologous recombination between the *ars1* sequence and its chromosomal counterpart; and *c*, integrated by homologous recombination between *his3* sequences. Letter and line designations are as in Fig. 1 with one addendum: B represents the predicted *Bam*HI endonuclease cleavage sites.



occurred when the transforming DNA contained the same *his3* allele as the recipient yeast strain (*his3*-532), in which case no *His*⁺ recombinants could be isolated. All the recombinants obtained were stable for both *His*⁺ and *Trp*⁺ and grew at the wild-type rate in minimal medium. Hybridisation analysis (as described above) indicated that the transforming DNA had integrated at the *his3* locus without detectable sequence alteration (data not shown). The frequency of recombination between the *his3*-38 allele (on the transforming plasmid) and the *his3*-532 allele (in the recipient) was roughly the same as the frequency observed when the two alleles were present in a heterozygous diploid yeast strain. In all respects, the integration of *ars1*-containing hybrid molecules seems to be a simple consequence of mitotic recombination.

The organisation of DNA replication in *Saccharomyces cerevisiae* is similar to that of other eukaryotes. Fibre autoradiography and electron microscopy studies indicate that each chromosome is divided into several replication units and the initiation of DNA synthesis for each unit is rigidly controlled²⁰⁻²³. The ability of *ars1* sequences to integrate has implications for this control. We have shown the DNA sequences

containing a replicator can stably integrate at other loci. As *ars1* is located near the centromere of chromosome IV and *his3* maps on the left arm of chromosome XV, the two loci are probably in different replication units. The observation that *ars1* can integrate at *his3* indicates that either initiation of DNA replication at *ars1* is suppressed in its new position or translocation of a second replicator simply creates a new functional replication unit.

Multimeric forms of *ars1*

Plasmids containing *ars1* have also been integrated in the *trp1*-*ars1* region near the centromere of chromosome IV (data not shown). As such a recombination event creates a tandem duplication, it suggests that multimers of a chromosomal replicator function normally. To investigate this further, we examined the *ars* phenotype of monomer, dimer and trimer YRp7.

When YRp7 is propagated in a recombination-proficient bacterial host, monomer, dimer and trimer forms of the plasmid arise. Each of the three forms of the plasmid DNA was purified

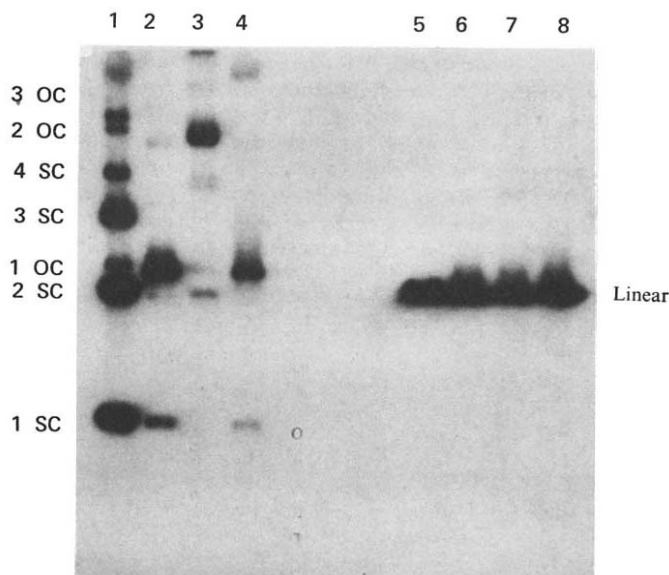


Fig. 3 Autonomous replication of monomer, dimer and trimer YRp7. Plasmid DNA purified from *trpC9830* (*Rec⁺*) carrying YRp7 was used to transform KM601, a *recA⁻recB⁻* *E. coli* strain (gift of Kevin McEntee). Plasmid DNA was rapidly isolated from individual tetracycline-resistant transformants and its size was assessed by agarose gel electrophoresis. Transformants were then picked that contained predominantly monomer, dimer and trimer YRp7. The plasmid DNAs were purified and analysed by restriction endonuclease digestion and agarose gel electrophoresis. Each sample contained some larger multimers, but neither the dimer nor the trimer DNA preparations contained detectable amounts of monomer plasmid and all were free of any gross alterations of the *ars1* sequences (data not shown). Monomer, dimer or trimer YRp7 DNA was used to transform D13-1A to *Trp⁺*. DNA from the transformants was purified, electrophoresed in a 0.4% agarose gel, transferred to nitrocellulose and hybridised to ³²P-labelled pBR322 DNA as described in Fig. 2 legend. DNA in lanes 1-4 was uncut while the DNA samples in wells 5-8 were digested with *Bam*HI restriction endonuclease. Lanes 1 and 5 contained 1 µg of wild-type yeast DNA and 2 ng each of purified monomer, dimer and trimer YRp7 DNA. The various multimeric forms are designated by the numbers 1, 2, 3 and 4 (for monomer, dimer, trimer and tetramer) while SC and OC distinguish supercoiled from nicked, open circular DNA. Lanes 2 and 6 each contained 1 µg of DNA purified from D13-1A transformed to *Trp⁺* by the YRp7 monomer form. DNA samples purified from D13-1A transformed by dimer and trimer YRp7 were electrophoresed in lanes 3, 7 and 4, 8 respectively.

by clonal isolation in a *recA⁻recB⁻* *E. coli* strain. Transformation of a recombination-proficient *trp1⁻* yeast strain to *Trp⁺* occurred at equal frequencies for monomer, dimer or trimer DNA (2,000–5,000 colonies per µg DNA). Strains transformed by each of the three DNAs had identical growth rates and were equally unstable mitotically. The structure of the transforming DNA in such strains was analysed (Fig. 3). Strains transformed with monomer and dimer YRp7 contained, respectively, monomer and dimer autonomously replicating supercoiled circles (lanes 2 and 3). Small amounts of other plasmid forms were detected (dimer in the monomer transformant as well as both monomer and tetramer in the strain carrying the dimer form). A strain transformed with trimer YRp7 DNA contained predominantly monomer plasmid (lane 4). It is unlikely that this isolate arose through transformation by contaminating monomer plasmid in the trimer DNA preparation (see Fig. 4 legend), so appearance of the monomer plasmid probably represents recombinational reduction of the multimeric form. The various circular forms in all three strains contained intact YRp7 sequences as judged by *Bam*HI endonuclease digestion which reduces all multimeric species to a single linear fragment (Fig. 2, lanes 5–8). Thus monomer and dimer YRp7 seem to be functionally equivalent. Some inter-conversion of multimeric forms occurs, probably by mitotic recombination in the yeast host cells.

A unique feature of *ars1* is that it is tightly linked to the centromere of chromosome IV. In fact, we considered the

possibility that the *ars* phenotype could be caused by a centromeric function. As described above, Sc4101 is capable of both stably integrating at other chromosomal loci and functioning in a dimeric state. If Sc4101 contained a centromere, both of these constructions would represent dicentric molecules which might be expected to undergo sequence alterations when propagated mitotically³⁰. As we detected no such alterations, we conclude that Sc4101 is unlikely to have full centromeric function. However, these experiments suggest tests for segregational activity which may lead to the identification of other yeast fragments that contain centromeric sequences.

Delimitation of *ars1*

Sc4101 contains two functions: *trp1* and *ars1*. To determine the position of the *ars1* sequences we have constructed several deletions of the 1.4-kilobase pair *Eco*RI-generated fragment. The experimental approach is shown in Fig. 4. Unique restriction endonuclease sites in Sc4101 were fused to unique sites in the pBR322 sequence, deleting parts of both Sc4101 and pBR322. Utilising both YRp7 and YRp7', it was possible to construct two pairs of complementary deletions; Yp411, Yp413 and Yp412, Yp414. Fragments containing the *ura3* or *his3* sequences (known to function when attached to an intact *ars1* element) were inserted into each of the four deletions. Only Yp413/*HIS3⁺* and Yp412/*URA3⁺* DNAs were capable of transforming *his3⁻* and *ura3⁻* yeast strains, respectively. Both transformed at high efficiency (again ~2,000 colonies per µg DNA). Yp412/*URA3⁺* had a completely normal *ars1* phenotype. Strains transformed with Yp413/*HIS3⁺*, however, had a greatly reduced growth rate, with a generation time of about 11 h, and an enhanced mitotic instability. Assuming that juxtaposition of the *ura3* or *his3* sequences does not effect *ars1* function, we conclude that the *ars1* sequence is fully contained within the 850 base pairs between the *Eco*RI and *Hind*III cleavage sites and deletion of the 200 base pairs between the *Hind*III and *Pst*I sites is an *ars1* mutation.

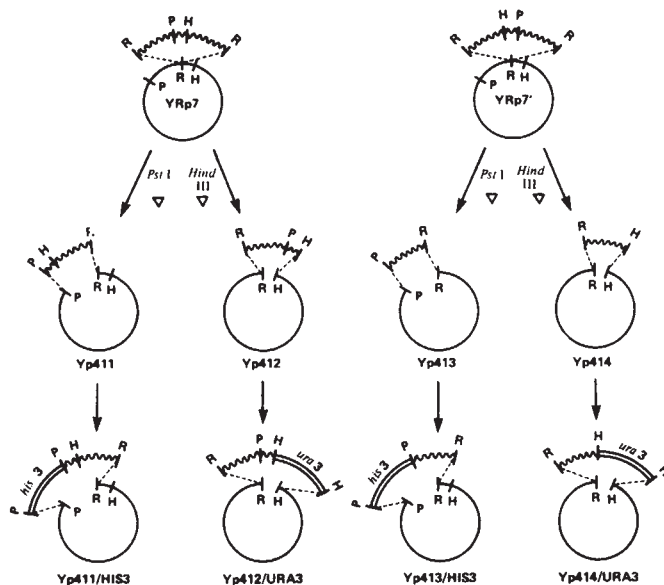


Fig. 4 Deletion analysis of Sc4101. Both *Pst*I and *Hind*III restriction endonucleases have unique cleavage sites in Sc4101 and pBR322. Digestion followed by circularisation and ligation (as described in Fig. 1) results in deletion of pBR322 and yeast sequences. The plasmids in the second line, derived in this manner from YRp7 and YRp7', were identified by selecting the appropriate drug resistance after transformation of *E. coli*. Yeast structural genes were then inserted at the now unique *Pst*I or *Hind*III cleavage sites. The *his3*-containing yeast DNA fragment (Sc2710) was prepared by *Pst*I digestion of pGT2-Sc2605 (ref. 9) while the *ura3* fragment (Sc2904) originated from *Hind*III cleavage of λ590-Sc2710 (ref. 12). The resulting plasmids shown in the second line were identified by selection for complementation of *hisB* and *pyrF* lesions and for the appropriate drug resistances following transformation of the *E. coli* strains *hisB463* (ref. 28) and MB1000 (ref. 12). The structures of all of the molecules shown were confirmed by restriction endonuclease digestion and agarose gel electrophoresis. Line drawings and letterings are as described in Fig. 1 legend and text.

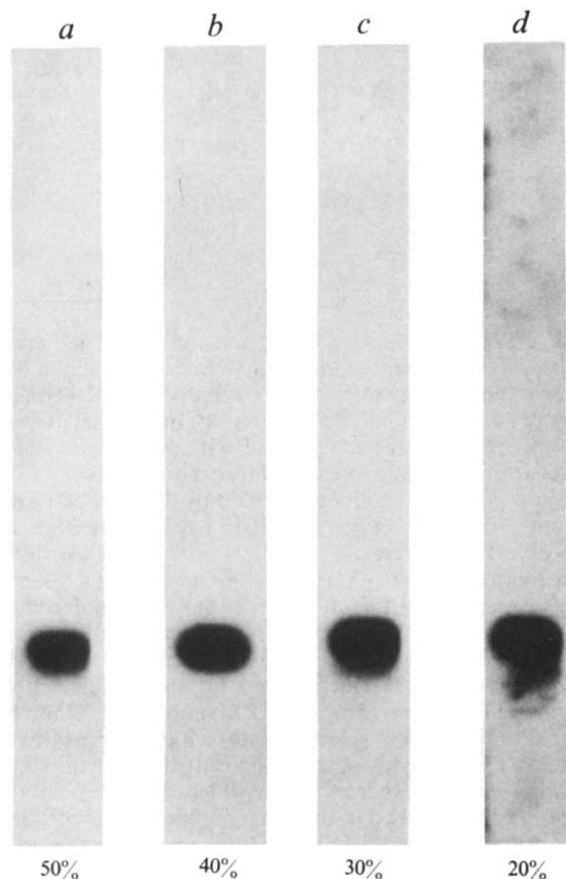


Fig. 5 Yeast sequences homologous to *ars1*. Yeast DNA was digested with *EcoRI* endonuclease and electrophoresed in a 0.6% agarose slab gel. The resulting *EcoRI* restriction spectra were transferred to nitrocellulose and then cut into four strips. After *EcoRI* digestion of YRp7 and gel electrophoresis, Sc4101 was purified by binding the DNA to a glass fibre filter (M. W. McDonnell and R. W. D., in preparation). The isolated fragment was then nick-translated. 10^6 c.p.m. of ^{32}P -labelled Sc4101 DNA was hybridised to each nitrocellulose strip in 5XSSPE (0.75 M NaCl, 50 mM NaPO₄, 5 mM EDTA), 0.2% SDS with 50% (v/v), 40%, 30% and 20% formamide (strips a, b, c and d, respectively). Each strip was washed in its own hybridisation conditions and autoradiographed. Long exposures were used to try to detect faint bands.

Are sequences homologous to *ars1* found elsewhere in the yeast genome?

Using measured values for the DNA elongation rate and the total size of the yeast genome, it was estimated that a minimum of 70 initiations must occur if the cell is to replicate its DNA fully during the S phase of the cell cycle²². These other chromosomal replicators may have DNA sequences in common with *ars1*.

In an attempt to detect *ars1* sequences present elsewhere in the yeast genome, ^{32}P -labelled Sc4101 DNA was hybridised to a restriction endonuclease spectrum of yeast DNA. The hybridisation conditions were varied to allow fragments with a significant proportion of mismatched base pairs to anneal. As shown in Fig. 5, even in the least stringent conditions (Fig. 5d), only the single homologous 1.4-kilobase pair fragment hybridised to Sc4101. The conditions used would have required at least 75% homology to *ars1* (over a 500-base pair region) for hybridisation to occur. Alternatively, other yeast replicators probably have less than 20 sequential base pairs in common with *ars1*.

The usefulness of hybrid molecules containing *ars1*

We have previously discussed some of the uses of plasmids containing the Sc4101 fragment⁹. Results reported here point out additional applications. Vectors that contain *ars1* (which we

shall call 'YR vectors') can be maintained in yeast cells in two easily distinguishable states. Although autonomously replicating in selective conditions, YR transformants are mitotically unstable. In this state, the supercoiled YR DNA can be easily re-isolated (D.T.S., K.S. and M. Thomas, unpublished observations). In addition, stable transformants with integrated YR sequences can then be easily obtained at high frequency. In this second state, the location of the integrated DNA can be determined physically (ref. 9 and above) or by simple genetic analysis (see ref. 11). Such linkage analysis could determine the chromosomal location of a yeast DNA fragment that had been cloned in a YR vector. Integrated YR vectors can also be used for directed genetic manipulation, wherein a sequence is replaced by an altered but homologous counterpart through an integration-excision mechanism^{9,12}.

In addition to its uses as a vehicle for clonally isolating DNA in yeast, YRp7 will facilitate studies of yeast DNA replication in several ways. First, continuation of the preliminary genetic analysis described here will determine the sequences essential for the functioning of a eukaryotic chromosomal replicator. Second, other functions that control *ars1* replication may be identifiable by genetic or biochemical techniques; for example, several mutants that are temperature-sensitive for the initiation of DNA synthesis have been characterised²⁴; their effects on *ars1* replication could be studied in detail. Third, the supercoiled YRp7 plasmid could be used (either with or without its chromatin protein complement) as a template for *in vitro* DNA replication systems.

The high frequency of transformation associated with *ars1* suggests an easy method for the isolation of other chromosomal replicators. Random fragments of DNA can be inserted into a yeast vector that transforms only at low efficiency. Transformation of a yeast strain by the hybrid molecules will result in an enrichment for those fragments which allow autonomous replication. Studies of such *ars* loci isolated from yeast and other organisms are in progress.

The characterisation of a chromosomal replicator represents the first step towards the goal of constructing an operational eukaryotic chromosome. The isolation of other elements that control replication and proper segregation will not only elucidate the mechanisms of chromosome function but will provide the means for determining how chromosome structure affects gene expression.

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Ca²⁺-sensitive gelation of actin filaments by a new protein factor

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Two protein factors which bind to, and induce gelation of, actin filaments were purified from Ehrlich tumour cells. Filamin induced Ca²⁺-insensitive gelation, whereas a new protein factor ('actinogelin') was found to induce Ca²⁺-sensitive gelation.

FOR a better understanding of the cellular functions of actin filaments more information on the properties of actin accessory proteins is needed. Actin-related gelation of cell-free extracts prepared from amoebae¹⁻³, mammalian cells⁴⁻⁶ and sea urchin eggs⁷ provides an excellent system for studies on actin accessory proteins, especially on the regulation of their interaction with actin filaments.

Involvement of Ca²⁺ in the regulation of microfilament function is possible, since intracellular Ca²⁺ at micromolar concentration is known to influence processes such as cell motility⁸, chemotaxis⁹, secretion^{10,11} and fertilisation¹². Studies on reversible inhibition of actin-related gelation by micromolar concentrations of Ca²⁺ in a large variety of cells^{3,6,7-13} further support this hypothesis. Possible involvement of Ca²⁺ in HVJ(Sendai virus)-induced cell fusion^{6,14-16}, chemically induced cell fusion¹⁷ and fusion of myoblast¹⁸ has also been reported.

Ca²⁺-insensitivity of filamin(actin binding protein)-dependent gelation

Since filamin (or a protein similar to actin binding protein of Stossel and Hartwig³) is concentrated in gels together with actin from Ehrlich tumour cell extracts (Fig. 1a, b), the role of filamin in Ca²⁺-sensitive gelation was studied. As shown in Fig. 1c, filamin purified from Ehrlich tumour cells by a combination of the methods described for actin binding protein of macrophage¹⁴ and for smooth muscle filamin^{19,20}, gave a single band by SDS-gel electrophoresis. The gel formed by mixing this protein with skeletal muscle actin consisted of only filamin and actin (Fig. 1d). Degree of gelation measured by pelleting of protein by centrifugation at 49,000g for 10 min, is dependent on the amount of filamin added but is not appreciably affected by the presence of either Ca²⁺ or EGTA (Fig. 2). Similar insensitivity towards Ca²⁺ in a purified and reconstituted system was recently reported for 'actin binding protein'-dependent gelation of rabbit alveolar macrophage extracts²¹ and '57 K dalton and 220 K dalton protein'-dependent gelation of sea urchin egg extracts²². As a result, the search for calcium-sensitivity conferring factor(s) is under way in several laboratories^{6,23,24}.

Purification of a Ca²⁺-sensitive gelation factor (actinogelin)

During a search for a factor which confers Ca²⁺-sensitivity on filamin-related gelation of muscle actin, a protein fraction was obtained from Ehrlich cell extracts which, surprisingly, did not contain filamin but did produce gel on mixing with rabbit skeletal muscle actin, provided that 1 mM EGTA is included in the reaction mixture. No requirement for filamin was observed for gelation by this protein factor as described below. As shown in Fig. 3, the degree of gelation assayed by the centrifugation method is proportional to amounts of DE32 eluate fraction added to skeletal muscle actin. Further purification of the protein fraction on hydroxyapatite column showed that a pro-

tein, of MW 115,000 on SDS-polyacrylamide gel electrophoresis, was concentrated in an active fraction (fraction b) and the same band was also selectively concentrated with actin in gel fraction pelleted by centrifugation (Fig. 4). In contrast, there was no co-precipitation of such a band with actin from reaction mixtures containing inactive fractions (fractions a and c).

This new gelation factor was purified by the method outlined below until the product gave a single band on SDS-gel electrophoresis (Fig. 1e) (see legends to Figs 1 and 4 for details). Cell-free extracts were obtained by the method described previously⁶ except that Tris-maleate buffer was replaced with 20 mM imidazole-HCl buffer (pH 7.0). After adjusting KCl concentration of the extract to 0.6 M, the extract (190 ml, about 5,700 mg protein) was fractionated by ammonium sulphate precipitation (between 15% and 35% saturation). One unit is defined as the amount of gelation factor which precipitates 1 mg of actin in a Ca²⁺-sensitive process on centrifugation at 49,000g for 10 min (control experiments in which the same actin preparation was centrifuged in the same medium were performed, and the value obtained was subtracted from the experimental values). The same ammonium sulphate fractionation cycle was repeated, and the resultant fraction was dialysed and then clarified by centrifugation. The supernatant (630 mg protein, 189 U) was applied on DE32 column, and an active fraction was eluted with 0.4 M KCl (77 mg protein, 215 U). Further purification was achieved by hydroxyapatite column chromatography and the active fraction (16 mg protein, 110 U) was applied on and eluted from Sepharose 6B column. On electrophoresis, about one-third of the fractions obtained (2.5 mg protein, 24 U) were judged pure (Fig. 1e). For higher recovery, fractions containing appreciable activity were pooled (6.2 mg protein, 57 U), and some of this was further purified by chromatography on DE32. Since filamin interferes with the assay, in crude preparations the factor cannot be determined accurately. However, a purification of ~30-fold was achieved with 30% recovery from the second ammonium sulphate fractionation step to Sepharose 6B gel filtration step. From the final yield of the protein (about 0.1% of crude extract), the content of the Ca²⁺-sensitive gelation factor in crude extracts is calculated to be >0.3%. This protein is tentatively named as 'actinogelin' to avoid confusion with the other actin binding proteins. Gel reconstituted from skeletal muscle actin and actinogelin was found to be composed of actinogelin and actin (Fig. 1f).

Thus two gelation systems which differ in sensitivity towards micromolar concentrations of Ca²⁺ could be reconstituted from skeletal muscle actin and protein fractions which were purified from the same cell-free extracts. To establish whether these fractions can be detected in crude systems, SDS-gel electrophoretograms of crude cell-free extracts in the presence and absence of EGTA were compared (Fig. 1a, b). Bands corresponding to filamin, actinogelin and actin were concentrated in the gel. 'Actinogelin' and 'filamin' are confirmed by co-electrophoresis of the gel fraction with purified proteins. Protein bands of similar electrophoretic mobility (MW ~100,000-120,000 K) can be detected in published electrophoretograms of crude gel fractions of HeLa cells²⁵, leukocyte extracts²⁶ and amphibian cell extracts¹³, although they were not mentioned in the texts. Thus it seems possible that two different gelation factors might co-exist in several cells other than Ehrlich tumour cells.

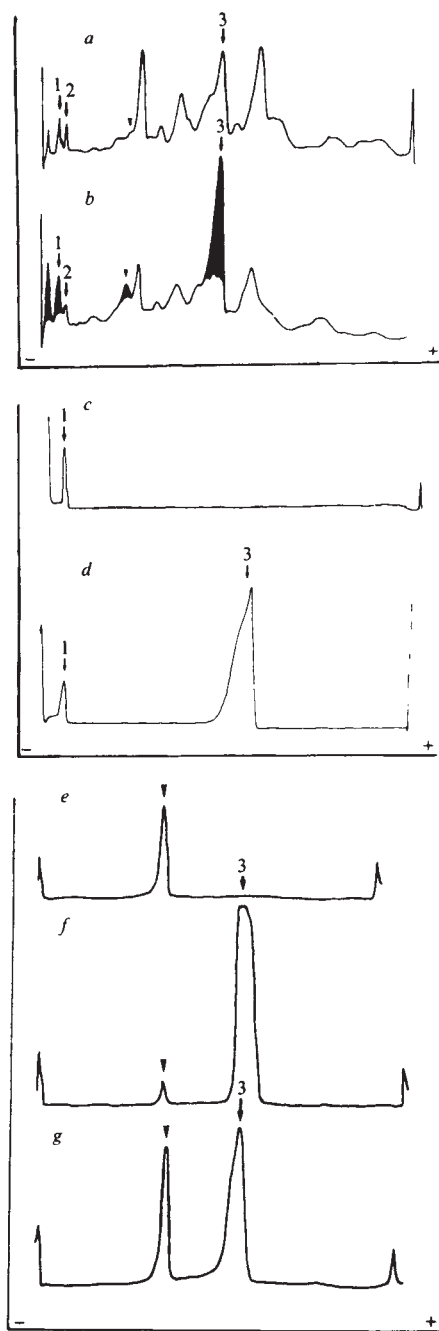


Fig. 1 SDS-polyacrylamide gel electrophoretograms of crude and purified gelation factors and gels. *a*, Crude extract; *b*, gel formed from crude extract, protein bands which were concentrated in the gel compared to the extract are shown in black. *c*, Filamin purified from Ehrlich tumour cells as described in Fig. 2 legend. *d*, Gel formed by reconstitution of 340 µg actin and 20 µg of filamin. *e*, Purified actinogelin (Sephacryl 6B fraction), 10 µg. *f*, Gel formed by reconstitution of 352 µg actin and 21 µg of pure actinogelin and centrifuged at 49,000g for 10 min. The amount of total protein applied to the gel column was 30 µg. *g*, Precipitate formed by reconstitution of 40 µg actin and 40 µg of pure actinogelin, followed by centrifugation at 140,000g for 3 h. Amount of total protein applied to the gel column was 30 µg. Pure actinogelin was obtained by Sepharose 6B column chromatography of the hydroxyapatite eluate fraction. The pooled fraction obtained as described in Fig. 4 legend was concentrated to 1.0–1.5 ml and applied to a column of Sepharose 6B (1.2 × 95 cm) equilibrated with 20 mM Tris-HCl (pH 7.6) containing 50 mM sodium chloride, 0.1 µg ml⁻¹ pepstatin and 1 mM sodium azide. Elution was carried out by the same buffer and 1.2-ml samples were collected. Actinogelin was eluted from the column as a major peak with some tailing material(s). The main fractions whose purity was checked by SDS-polyacrylamide gel electrophoresis and found to be homogeneous were pooled and used as pure actinogelin preparation. Bands 1, 2 and 3 correspond to filamin, myosin heavy chain and actin, respectively. Actinogelin is marked by an arrowhead. Acrylamide at 5.6% was used.

Characteristics of actinogelin-dependent gelation

The effects of free Ca^{2+} on the extent of gelation in the actinogelin-dependent system were almost identical to the effects in crude extracts—gelation was inhibited by micromolar concentrations of free Ca^{2+} (Fig. 5). Furthermore, rapid solation of the formed gel by increasing free Ca^{2+} occurred at similar Ca^{2+} concentrations in the two systems⁶. Gelation activity of purified actinogelin is similar to or higher than that of pure filamin in Ehrlich tumour cells on weight basis. Since the MW of native actinogelin is not yet known, we cannot calculate specific gelation activity. But a molar ratio of ~1:100 of either filamin or actinogelin (calculated as dimer) could induce detectable gelation of skeletal muscle actin.

The maximum ratio of binding of actinogelin to F-actin was estimated for a fixed amount of actin in the system (40 µg in 80 µl), with increasing amounts of actinogelin added to it, and by applying centrifugation at 140,000g for 3 h to precipitate F-actin. Resultant pellets were analysed by SDS-gel electrophoresis (Fig. 1g). A linear increase of actinogelin recovered in the pellet fractions was observed at up to 20 µg actinogelin per tube, but no further increase was detected when the actinogelin level was raised to 40 µg: no appreciable actinogelin (0–3 µg) was found in the supernatant fraction when up to 20 µg actinogelin was present in the system, whereas at 40 µg about 21 µg of the added actinogelin was recovered in the supernatant. Thus it is unlikely that the preparation is contaminated with protein which lacks actin binding ability. No measurable precipitation of actinogelin was observed in control experiments without actin. Assuming that actinogelin is mainly present as the dimer (preliminary observation), we estimate the molecular ratio of actin to actinogelin at maximum binding to be ~10–12.

Possible participation of Ca^{2+} -activated protease in Ca^{2+} inhibition of gelation²⁷ was checked by measuring the reversibility of the inhibition. Gelation mixture containing actinogelin and actin was preincubated in the presence of 50 µM Ca^{2+} for 60 min at 20 °C, and EGTA was added to a final concentration of 400 µM to decrease the free Ca^{2+} concentration. The degree of gelation of the treated system was identical with that of unpreincubated control. Thus, irreversible destruction of reaction components by a Ca^{2+} -activated protease could be excluded. The effect of Ca^{2+} on G to F transformation of skeletal muscle actin was measured by determining the amounts of F-actin pelleted at 140,000g for 3 h. No appreciable difference in the amounts of F-actin pelleted was observed between centrifugations performed in the presence or absence of Ca^{2+} in the experimental conditions used.

The possibility that small MW Ca^{2+} -binding protein similar to Ca^{2+} -dependent modulator protein^{28,29} of non-muscle cells or leiotonin C of smooth muscle³⁰ might be present in actinogelin preparations and cause Ca^{2+} -sensitivity seems unlikely for the following reasons: (1) An actinogelin preparation purified to the Sepharose 6B step was passed through a Sephacryl S-200 column equilibrated with a medium containing 1 mM EGTA and 0.8 M KCl to facilitate dissociation of any Ca^{2+} -modulator protein that may have been present. But, no decrease in gelation activity and Ca^{2+} -sensitivity of the actinogelin preparation was observed after such treatment. (2) An actinogelin preparation was rechromatographed on DE32 in a medium containing 1 mM EDTA which was used for separation of acidic leiotonin C from leiotonin A³⁰, but no evidence for separation of highly acidic protein from actinogelin was obtained.

Semiquantitative densitometry of SDS-gel electrophoretograms of gel pellets showed actinogelin recover in the pellet fraction decreased in the presence of 50 µM Ca^{2+} to less than one-third of that of control with 0.5 mM EGTA. Whether this decreased binding of actinogelin to F-actin is sufficient to explain inhibition of gelation by Ca^{2+} , or whether a change in oligomeric structure such as a dimer to monomer conversion induced by Ca^{2+} must be introduced to explain the inhibition is not yet clear.

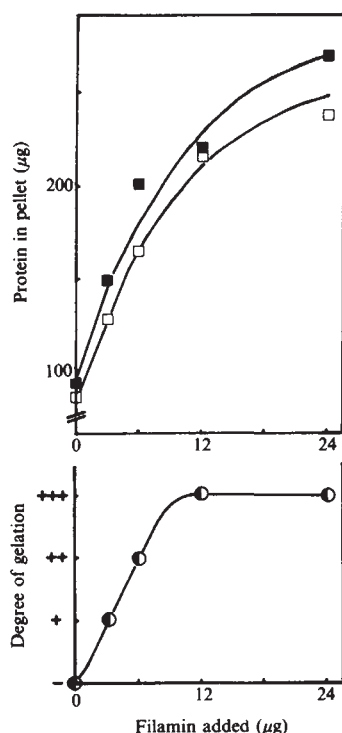


Fig. 2 Effects of Ca^{2+} on filamin (actin binding protein)-induced gelation of rabbit skeletal muscle actin. Reaction medium consisted of 10 mM imidazole-HCl buffer (pH 7.2), 50 mM potassium chloride, 1 mM magnesium chloride, 2.0 mg ml⁻¹ of rabbit skeletal muscle actin containing ATP at a final concentration of 65 μM , and either 1.15×10^{-4} M CaCl_2 (■) or 5×10^{-4} M EGTA and 6.5×10^{-5} M CaCl_2 (□). Filamin was purified from Ehrlich tumour cells as follows. Cells resuspended in two volumes of an extraction medium containing 0.34 M sucrose, 1 mM ATP, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulphonylfluoride (PMSF), 0.1 $\mu\text{g ml}^{-1}$ pepstatin, and 20 mM imidazole-HCl (pH 7.0 at 25 °C), were disrupted by a cell disruption bomb (Purr) as described previously⁶. The disrupted cells were centrifuged at 30,000 r.p.m. for 1.5 h in a Hitachi 55P ultracentrifuge with a RP30 rotor at 2° to 4 °C and the resultant clear supernatant was collected. As the first step, filamin was extracted from gel. After addition of 1 mM MgCl_2 , the cell extracts were warmed to 25 °C for 1 h and then resultant gels were sedimented at 40,000g for 10 min in a Sorvall SS-34 rotor. The pellets were washed twice with 0.1 M KCl in 20 mM imidazole-HCl (pH 7.0) and resuspended in one-fifth of the original volume of a medium containing 0.6 M KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 0.1 $\mu\text{g ml}^{-1}$ pepstatin and 10 mM imidazole-HCl (pH 7.0). The suspension was left to stand in a cold room (4 °C) with stirring overnight to release filamin from insoluble materials. Insoluble materials containing F-actin were removed by centrifugation at 100,000g for 3 h at 4 °C. The supernatant was collected and subjected to the second step of purification. Saturated ammonium sulphate solution with pH adjusted to 7.0 was added to the supernatant to give 15% saturation. After removing the resultant precipitate, the supernatant was brought to 35% saturation by adding saturated ammonium sulphate solution. The pellet was collected and suspended in 0.6 M KI solution (containing 0.6 M KI, 20 mM $\text{Na}_2\text{S}_2\text{O}_3$, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 0.1 $\mu\text{g ml}^{-1}$ pepstatin, 10 mM imidazole-HCl pH 7.0). The ammonium sulphate fractionation (15–35% saturation) was repeated, and the pellet was suspended in 1–2 ml of the 0.6 M KI solution. After standing for 30 min at 4 °C, the suspension was applied to a 1.6×83 cm column of Sepharose 4B equilibrated with a low ionic strength buffer (2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM CaCl_2 , 0.5 mM 2-mercaptoethanol, pH adjusted to 8.0), and eluted with the same buffer at a flow rate of 6 ml h⁻¹. Filamin was eluted as a major peak. Peak fractions were checked for purity by SDS-polyacrylamide gel electrophoresis, and fractions found to be homogeneous were pooled and used as pure filamin. If necessary, fractions with slight contamination were further purified by DEAE-cellulose (Whatman DE32) column chromatography. Filamin was eluted from the DE32 column by stepwise elution between 0.1 M and 0.15 M KCl dissolved in 20 mM Tris-HCl buffer (pH 7.6). Purified filamin was added to the gelation system as indicated to a final volume of 0.2 ml. Incubation was for 60 min at 20 °C. The degree of gelation was assessed by tilting tubes containing gels as described previously⁶, either in the presence of Ca^{2+} or EGTA (○). Gelation was quantitated by measuring amounts of protein pelleted by centrifugation at 49,000g for 10 min at 15 °C. Pellets were rinsed once by the reaction medium, then the amounts of protein were measured by the method used for the precipitate⁴³.

The role of actinogelin and filamin in gelation of crude extracts

Since at least two different gelation systems were found in Ehrlich tumour cell extracts, the relationship between these systems was studied. In the presence of a sub-optimum concentration of actinogelin and various amounts of filamin together with actin, gel formation is partially sensitive to Ca^{2+} at low concentrations of filamin. The extent of gelation is partially additive when EGTA is present in the medium, whereas in the presence of 50 μM Ca^{2+} it is almost identical with that of filamin alone. At higher concentrations of filamin, however, gelation becomes insensitive to Ca^{2+} (Fig. 6). Thus, the involvement of actinogelin in determining Ca^{2+} -sensitivity of the crude system cannot be unequivocally established until a quantitative determination can be made. But, quantitation of filamin and actinogelin in crude extracts is rather difficult, and effects of the other proteins which bind to actin, such as myosin and tropomyosin, on interaction of any of the gelation factors cannot be excluded. For example, heavy meromyosin inhibits sedimentability of filamin without appreciably affecting that of actin²⁵, and filamin was found to inhibit actin activation of heavy meromyosin ATPase³¹. Furthermore, the possible role of other regulatory systems has to be considered. Some of the studies on gelation of crude cell-free extracts which may shed light on this problem are discussed below.

Gelation of some ascites tumour cell extracts is enhanced by tryptic digestion of the extracts. Close examination of the resultant gel from the treated sample revealed that no filamin-like component is concentrated in the gel, whereas that obtained from untreated control contained a filamin-like component³². Although Ca^{2+} -sensitivity was not tested (EGTA was included in the reaction mixture), this result suggests the presence of a gelation factor which is different from filamin. On the other hand, Brotshi *et al.*²¹ surveyed gelation factors of rabbit alveolar macrophage extracts and found that the main component of gelation of the extracts is high MW 'actin binding protein'. But, as they did not include Ca^{2+} chelators in the reaction medium, no Ca^{2+} -sensitive gelation factor would have been detected.

Thus actinogelin appears to play an important part in the Ca^{2+} -sensitive gelation of the crude extract of Ehrlich tumour cells for the following reasons: (1) In crude extracts actinogelin becomes concentrated in the gel; (2) the concentrations of Ca^{2+} required for inhibition of gelation are almost identical in crude and reconstituted systems; (3) no other fractions from the crude extract would either induce Ca^{2+} -sensitive gelation or confer Ca^{2+} -sensitivity to filamin-dependent systems; (4) the amounts of actinogelin recovered in the purified fraction were sufficient to explain gelation of the crude extracts.

Comparison of actinogelin with the other actin-accessory proteins

There are several proteins which bind to either actin filaments or monomeric actin in animal cells. Those which bind to filamentous actin and cross-link between actin filaments could form actin-related gels. Filamin (actin binding protein) can form

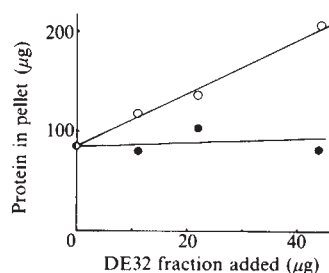


Fig. 3 Reconstitution of Ca^{2+} -sensitive gelation with rabbit skeletal muscle actin and a crude fraction partially purified from Ehrlich tumour cell extracts by DE32 chromatography. ○, In the presence of 1 mM EGTA and 9×10^{-5} M CaCl_2 ; ●, with 9×10^{-5} M CaCl_2 added.

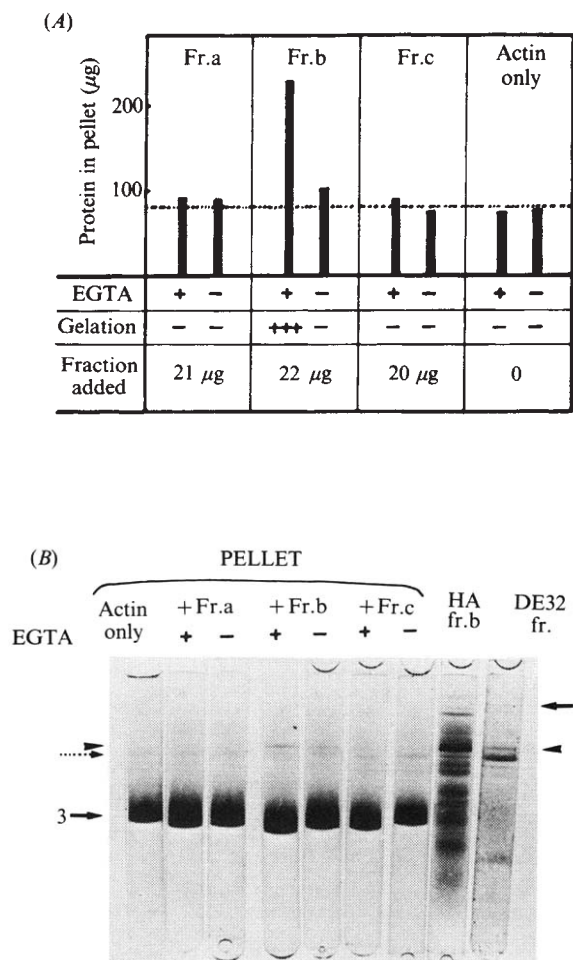


Fig. 4 Purification of actinogelin by hydroxyapatite chromatography. Actinogelin was purified to DE32 step as follows: Cell-free extracts were prepared as described in Fig. 2 legend. After adjusting KCl concentration of the extract to 0.6 M, the extract was fractionated by ammonium sulphate (between 15% and 35% saturation). The resultant pellet was dispersed with a buffer containing 10 mM imidazole-HCl (pH 7.0), 0.1 mM KCl, 2 mM $MgCl_2$, 0.1 mM PMSF and $0.1 \mu g ml^{-1}$ pepstatin and then dialysed overnight against the same buffer. The dialysed solution was adjusted to a final concentration of KCl of 0.6 M, and was spun at 100,000g for 3 h, and clear supernatant was fractionated again by ammonium sulphate between 15% and 35% saturation. The resultant pellet was dispersed with a buffer containing 20 mM imidazole-HCl (pH 7.2), 0.1 mM PMSF and $0.1 \mu g ml^{-1}$ pepstatin and then dialysed overnight against the same buffer. Insoluble materials were removed by centrifugation at 40,000g for 10 min in a Sorvall SS-34 rotor and the clear supernatant was applied to a 3×15 cm column of DE32 which was equilibrated with 20 mM imidazole-HCl buffer (pH 7.2). The column was washed with 100 ml of 20 mM imidazole buffer and subsequently washed with 400 ml of 20 mM imidazole buffer containing 0.2 M KCl, and then actinogelin was eluted with 400 ml of 0.4 M KCl in 20 mM imidazole buffer. The fractions eluted with 0.4 M KCl were dialysed against 10 mM potassium phosphate (pH 6.8) and applied to a 2×6.5 cm column of hydroxyapatite. After washing the column with 70 ml of 50 mM potassium phosphate (pH 6.8), elution was carried out with a linear gradient prepared with 60 ml of 50 mM potassium phosphate and 60 ml of 0.25 M potassium phosphate (pH 6.8) at a flow rate of 10 to 12 $ml h^{-1}$. Actinogelin was eluted as a major peak between 0.1 and 0.2 M potassium phosphate. Fractions with an appreciable actinogelin content on SDS-polyacrylamide gel electrophoresis were pooled as fraction b. Fractions just preceding the actinogelin peak were collected as fraction a, and those following the fraction b were used as fraction c. *a*, The gelation activity of the three fractions designated a, b and c was measured as described in Fig. 2 legend. Only fraction b has the ability to reconstitute Ca^{2+} -sensitive gelation. *b*, Electrophoretograms of purified fractions and gels. Although several protein bands are concentrated in fraction b compared with DE32 fraction, only a protein band (actinogelin) marked by an arrowhead ($\blacktriangleright$) is bound to actin. The band marked no. 3 is actin and the band marked by a dotted arrow is a contaminant of this particular actin preparation. The arrow marked no. 1 shows the migration position of filamin in the conditions used. The amounts of total protein applied to each acrylamide gel column were 25, 46, 42, 43, 42, 31, 31, and 13 μg , respectively from left to right. Acrylamide gel at 5.6% was used.

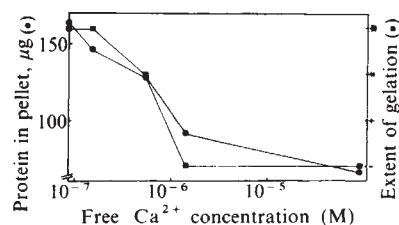


Fig. 5 Effect of free Ca^{2+} concentrations on the extent of gelation of a reconstituted system consisted of skeletal muscle actin and actinogelin. Reaction conditions were similar to those described for Fig. 2, except that 472 μg of actin and 150 μg of crude actinogelin (DE32 fraction) were used. Free Ca^{2+} concentrations were manipulated by an EGTA- Ca^{2+} buffer system⁴³ as described previously⁶.

Ca^{2+} -insensitive gels as described above, α -actinin and tropomyosin have been shown to bind to actin cables by a fluorescence technique³³, and α -actinin produced gel when added to F-actin^{34,35}, although this gelation was not sensitive to Ca^{2+} (K. Maruyama, personal communication). Mixing of F-actin with tropomyosin and troponin also produced transient gelation, but this gelation was not inhibited by Ca^{2+} , although reformation of gel from high temperature-dissociated samples was inhibited by Ca^{2+} (ref. 36). The other characteristics of troponin-related gelation³⁷ are quite different from those of actinogelin-related gelation described in this article, and there is no evidence for the presence of troponin in non-muscle cells³⁸. Kane's 58,000 MW protein isolated from sea urchin eggs is required for actin bundle formation, and interestingly, there is another protein (MW 220,000) which binds to actin-58,000 MW protein 'bundles' and is required for gel formation in sea urchin eggs²². No Ca^{2+} -sensitivity was found in this reconstituted system, although crude extracts of sea urchin eggs formed a Ca^{2+} -sensitive gel⁷. Lower MW proteins (gelactins) have been implicated in actin-related gelation of *Acanthamoeba castellanii* extracts². Ca^{2+} or Mg^{2+} is required for this type of gelation. Condeelis and Taylor³ have reported Ca^{2+} -sensitive gelation of cell-free extract of *Dictyostelium discoideum*, and purification of gelation factor(s) is under way²⁴. It was found recently that microtubule associated proteins interact with actin filaments, although binding force between these proteins is rather weak, in other words, no co-precipitation of microtubule associated proteins with F-actin could be observed³⁹.

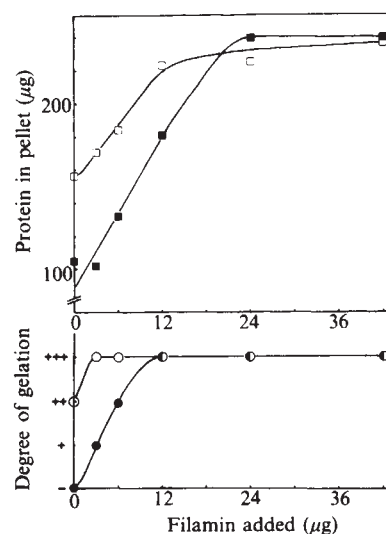


Fig. 6 Ca^{2+} -sensitivity of gelation reconstituted from skeletal muscle actin, actinogelin and filamin. Reaction conditions were similar to those described for Fig. 1, except that 400 μg of actin, 13.6 μg of actinogelin (hydroxyapatite fraction b) and the indicated amounts of filamin were used. $\square$, $\circ$, Experiments in the presence of 0.5 mM EGTA and 65 μM $CaCl_2$; $\blacksquare$, $\bullet$, in the presence of 115 μM $CaCl_2$.

Binding of a large amount of F-actin to clathrin (major protein of coated vesicles) was reported recently⁴⁰, but no effect of Ca^{2+} on this interaction is known. Thus, none of the gelation-inducing proteins reported previously has been identified as a Ca^{2+} -sensitive gelation factor, with the possible exception of a protein fraction reported in an abstract²³ which confers Ca^{2+} -sensitivity on actin binding protein-dependent gelation. The relationship between this protein and actinogelin is not yet clear, as a detailed report on Ca^{2+} -sensitivity conferring protein is not yet available. One difference is obvious, however—actinogelin alone may form a gel by interaction with F-actin, but the macrophage factor requires actin binding protein for gelation²³.

Micromolar concentrations of Ca^{2+} seem to exert at least two different effects on microfilament-dependent functions³⁸; one is the well established effect of Ca^{2+} on actomyosin ATPase and superprecipitation of actomyosin^{38,41} and the importance of this

type of regulation is well known. The possible importance of Ca^{2+} -regulation of actin-to-actin (or actin-to-actin binding proteins) interaction has received less attention. But, as is reported or quoted here, Ca^{2+} definitely affects actin-to-actin interaction reversibly at concentrations similar to those which activate actomyosin ATPase. And, the distribution of actin is known to change extensively in several cellular functions, such as virus-induced transformation, in non-muscle cells. Thus, those two types of regulation seem to be equally important in non-muscle cells.

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letters

Universal microwave radiation and extragalactic γ radiation

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The detection of an extragalactic component of celestial γ -radiation^{1,2} has stimulated the question of the possible contributions from external point sources, especially since the detection of γ rays from NGC4151 (ref. 3) and from 3C273 (ref. 4). Here we discuss the role of the inverse Compton effect (ICE) of relativistic electrons on universal microwave photons for the production of high energy γ rays from normal spiral galaxies.

It was estimated^{5,6} from the total galactic γ -ray luminosity of our Galaxy that the possible contribution from normal spiral galaxies to extragalactic γ radiation is between 4 and 100% of the total for energies > 100 MeV, 100% being reached if evolutionary effects within the involved spiral galaxies are taken into account⁶. The resulting flux from all involved external objects in the framework of a Friedman world model is given by^{6,7}

$$I(E_\gamma) \propto H_0^{-1} \cdot \int_0^{z_0} dz Q_\gamma [(1+z) \cdot E_\gamma] \cdot (1+\Omega \cdot z)^{-1/2} \cdot (1+z)^{-1} \quad (1)$$

where H_0 is the Hubble constant, Ω is the density parameter and $Q_\gamma(E_\gamma, z)$ is the total γ -ray emission of one object within the ranges of energy E'_γ , $E'_\gamma + dE'_\gamma$ and redshifts z , $z + dz$, which in this calculation is restricted to γ photons from normal spiral galaxies. To simplify the estimation of this contribution all normal spirals are assumed to be of the same age, t_g^0 , which is related to the value of z_0 up to which the redshift z has to be integrated by $t_g^0 = t_g(z=0)$, where the time scale is related to the scale of redshifts by

$$t_g = H_0^{-1} \cdot \int_z^{z_0} dz' (1+z')^{-2} \cdot (1+\Omega \cdot z')^{-1/2} \quad (2)$$

Here we compare the contribution of the ICE of relativistic electrons on universal microwave photons for the production of high-energy γ rays with those from other electromagnetic processes such as bremsstrahlung of relativistic electrons. To simplify the calculations we assume that the mean γ -ray emission of spiral galaxies has a diffuse origin and that the physical parameters which are relevant to these processes are equal to those in our Galaxy and do not change as these galaxies evolve,

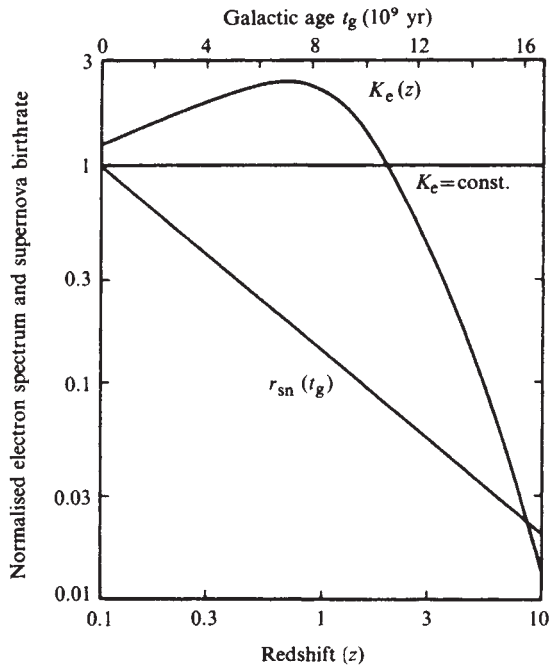


Fig. 1 Normalised supernovae birthrate $r_{\text{SN}}(t_g)$ (from ref. 10) and normalised electron spectrum $K_e(z)$ of high-energy electrons for the case of time-dependent electron sources and losses and for the case of constant galactic parameters. $\Omega = 0.08$; $H_0 = 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

except for the production rate of relativistic electrons which is believed to be associated with the birthrate of supernovae or pulsars, and with the cosmological evolution of the energy distribution and temperature T of the universal microwave radiation. Consequently, the cosmological change of the electron spectrum must also be considered. At present it is not possible to investigate this problem in more detail.

The two aforementioned processes are essentially influenced by the differential energy spectrum of relativistic electrons. As we wish to consider γ rays in the energy range around 100 MeV, for bremsstrahlung the corresponding electron energies are around 200 MeV and for ICE on microwaves around 200 GeV. In these two energy ranges the electron spectrum in our Galaxy is of the form

$$I(E) \propto E^{-p} \quad (3)$$

with two different values of p (refs 8, 9). The resulting differential source functions of γ photons from electron bremsstrahlung and ICE on microwaves are

$$Q_{\text{br}}(E_\gamma) \propto E_\gamma^{-p_1} \quad (4)$$

and

$$Q_{\text{MW}}(E_\gamma) \propto E_\gamma^{-(p_2+1)/2} \cdot T^{(p_2+5)/2} \quad (5)$$

respectively, with different values of p_1 and p_2 . The ratio of these two source functions in our Galaxy at $E_\gamma = 100 \text{ MeV}$ has been estimated as

$$r = Q_{\text{br}}(100 \text{ MeV}) : Q_{\text{MW}}(100 \text{ MeV}) \approx 40 : 1 \quad (6)$$

which has comparatively large uncertainties.

The temperature of the universal microwave radiation varies as $T(z) = T_0 \cdot (1+z)$ with $T_0 = 2.7 \text{ K}$; the corresponding photon energy density is $w_{\text{phot}}(z) = w_{\text{phot}}(0) \cdot (1+z)^4$. Therefore this increase of temperature would be expected to increase the production of γ rays due to ICE on microwaves with increasing redshift according to equation (5). The sources of the electrons may be supernovae or pulsars with a spectrum

$$q(E, t_g) = \bar{K} \cdot E^{-s} \cdot r_{\text{SN}}(t_g) \quad (7)$$

where $r_{\text{SN}}(t_g)$ is the supernovae birthrate, normalised to $r_{\text{SN}}(t_g^0) = 1$. Models on galactic evolution¹⁰ suggest that

$$r_{\text{SN}}(t_g) \propto \exp\{-t_g/t_c\} \quad (8)$$

with the characteristic time t_c of the order of $4 \times 10^9 \text{ yr}$. The increase of w_{phot} with increasing redshift leads to increasing energy losses $\dot{E} \propto w_{\text{phot}} \cdot E^2$ which changes the equilibrium electron spectrum. Taking into account that the electron lifetime in our Galaxy is of the order of 10^7 yr , the time scale of the change of the supernovae birthrate is large compared with the time scale of electron diffusion. The change of the electron spectrum is thus determined by a continuity equation for the differential number density of electrons, $N(E, t_g)$, in which energy losses and leakage are balanced by a source term

$$\frac{\partial}{\partial E} (E \cdot N(E, t_g)) + \frac{N(E, t_g)}{\tau} = \bar{K} \cdot E^{-s} \cdot r_{\text{SN}}(t_g) \quad (9)$$

with the time appearing as a parameter. At electron energies around 200 GeV which are essential for the ICE on microwaves, energy losses are dominated by synchrotron emission and inverse Compton scattering which are $\propto E^2$. Thus

$$\dot{E}(E, z) = \{c_1 + c_2 \cdot (1+z)^4\} \cdot E^2 = a(z) \cdot E^2 \quad (10)$$

The solution of equation (9) with respect to equations (8) and (10) expressed in terms of redshift, z , is

$$N(E, z) \propto f(E, z) \cdot E^{-2} \cdot \int_E^\infty dE' \cdot E'^{-s} \cdot \exp\{+1/\tau \cdot a(z)E'\} \quad (11)$$

with

$$f(E, z) = r_{\text{SN}}(t_g(z)) \cdot a^{-1}(z) \cdot \exp\{-1/\tau \cdot a(z) \cdot E\} \quad (12)$$

At high energies this expression reduces to

$$N(E, z) = K_e(z) \cdot E^{-(s+1)} \quad (13)$$

where $K_e(z)$ is given by $\bar{K} \cdot r_{\text{SN}}(t_g(z)) \cdot a^{-1}(z) \cdot (s-1)^{-1}$. $\bar{K}$ and s are adjusted to experimental data⁹ at $z=0$. Figure 1 shows the evolution of K_e for the case of z -variable energy losses and supernovae births in comparison to the local value which is normalised to unity. Also shown is the normalised supernovae birthrate as a function of galactic age (taken from ref. 10). For the 200-MeV electrons, the main energy losses are due to ionisation and bremsstrahlung¹¹, so the increase of the photon energy density of the microwaves has negligible influence on these electrons. To compare the contributions to extragalactic γ radiation by the two abovementioned processes, equations (4) and (5) are inserted into equation (1) to give

$$I_{\text{br}}(E_\gamma) \propto H_0^{-1} \int_0^{z_0} dz \cdot K_e'(z) \cdot E_\gamma^{-p_1} \cdot (1+z)^{-(p_1+1)} \cdot (1+\Omega z)^{-1/2} \quad (14)$$

$$I_{\text{MW}}(E_\gamma) \propto H_0^{-1} \int_0^{z_0} dz K_e(z) \cdot E_\gamma^{-(p_2+1)/2} \cdot T_0^{(p_2+5)/2} \cdot (1+z) \cdot (1+\Omega z)^{-1/2} \quad (15)$$

The evaluation of the integrals (14) and (15) can be made for three different models. H_0 is adopted to be $60 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and Ω is taken as 0.08. The actual age t_g^0 of normal spiral galaxies and the corresponding value z_0 of redshift are not well known and need not correspond to the present observational limit of $z_{\text{max}} \sim 3.5$ for quasars. Therefore, even higher values can also be used.

Model A: both the equilibrium galactic electron spectrum and the electron sources are considered to be constant during the whole integration period. In this case the contribution of ICE on microwaves (curve A_2 in Fig. 2) overtakes the bremsstrahlung contribution (curve A_1) at a redshift of $z \sim 5$. Consequently, the major part of extragalactic γ radiation from spiral galaxies would be due to the ICE on microwaves at high redshifts.

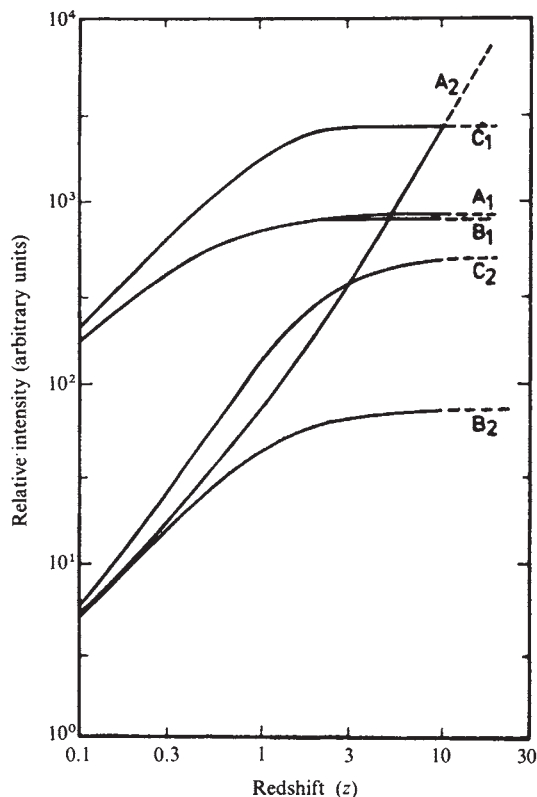


Fig. 2 Relative contributions of electron bremsstrahlung (index 1) and inverse Compton effect (index 2) from normal spiral galaxies to extragalactic γ radiation for the three evolution models A, B and C. $\Omega = 0.08$; $H_0 = 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$; $E_\gamma = 100 \text{ MeV}$.

Model B: the equilibrium electron spectrum at high energies is assumed to be dominated by energy losses $E \propto E^2$; the electron sources are still considered to remain constant. The resulting curves are B_1 and B_2 . The bremsstrahlung contribution changes to a very small degree in comparison with A_1 , but the ICE contribution is much lower than in model A. It shows a considerable flattening at high redshifts with a ratio $r_B = 11:1$.

Model C: electron sources as well as the equilibrium electron spectrum are assumed to be variable with galactic age. In this case the relative contributions by both processes (indicated by C_1 and C_2 in Fig. 2) increase in comparison with model B; a flattening of the curves is observed for redshifts greater than 4. Here the ratio of the contributions from the two processes at high redshifts is $r_C = 5.5:1$.

We conclude, therefore, that the contribution of γ photons from ICE on microwaves to extragalactic γ radiation is possibly more important than from local effects in our Galaxy; final results strongly depend on the evolution of the involved electron spectrum. In the case of no or weak evolution of the electron spectrum γ rays produced by ICE on microwaves can provide a considerable fraction of extragalactic γ radiation from spiral galaxies. Furthermore, the total galactic spectrum seems steeper at high redshifts than it is locally. These results depend on the choice of the cosmological parameters; if larger values for Ω are used, the integrals (14) and (15) change to a small degree.

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Is there a ring around the Sun?

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More than 300 years after the discovery of Saturn's rings, material rings or ring systems have been found in rapid succession around two of the major planets, Uranus¹ and Jupiter². With rings now known to exist around three of the four major planets, it is natural to assume that they also exist around Neptune. But what about the major object in the Solar System? Is the Sun itself surrounded by a ring of material? Although the planetary system already forms such a 'ring' system, what we have in mind here is the distinct possibility of a ring of rocky material lying no more than a few solar radii from the Sun, by analogy with the spatial configuration of the three planetary ring systems now known. We summarise here the evolutionary, physical, chemical and observational astronomical constraints that can be placed on such a hypothetical ring.

Models of planetary evolution in the early solar nebula give few definite clues pointing to the mass, orbital radius or size distribution of possible objects within the orbit of Mercury. In the absence of any reliable theoretical prediction, one can use the 'Titius-Bode Law' of planetary distances as an indicator of the orbital radius of a planet (or asteroid belt). Although there are several specific functional forms of this relation³, the one suggested by Blagg⁴ offers the closest fit to both the planetary orbital radii and the planetary satellite system orbital radii. Blagg's law predicts that the next orbit inside Mercury's orbit would lie at a distance of 0.175 AU from the Sun. This distance is marginally smaller than the radius of the synchronous orbit for a planet orbiting the sun, which lies at a distance $a_{\text{synchronous}} = (GMT_{\odot}^2/4\pi^2)^{1/3} \approx 0.177 \text{ AU}$, assuming $T_{\odot} = 27 \text{ d}$. Since the hypothetical planet lies within the synchronous orbit, tidal forces would tend to drag the object towards the Sun, until it breaks up at the Roche radius a_{Roche} . For fluid bodies, $a_{\text{Roche}} \approx 2.46(\bar{\rho}_{\odot}/\rho_r)^{1/3}R_{\odot}$. For material of density $\rho_r \approx 2 \text{ g cm}^{-3}$ (for example, a refractory material such as graphite) one finds $a_{\text{Roche}} \approx 2R_{\odot}$ and for refractory (Ca, Al) silicates of $\rho \approx 3.5 \text{ g cm}^{-3}$ the Roche limit moves inwards to $\sim 1.6R_{\odot}$. Such an argument naturally gives rise to a ring system about the Sun analogous to those found about the major planets. Indeed, a similar evolution has been suggested for the origin of the rings of Uranus^{5,13}. It is inapplicable, however, in the present case. The time scale τ for a planet of mass m , radius A and initial orbital radius r_0 to spiral into the Sun (mass M and relative energy dissipation rate Q) by tidal drag is

$$\tau \approx \left(\frac{4}{117} \right) Q r_0^{13/2} (M/G)^{1/2} A^{-5} m^{-1} \text{ (seconds)} \quad (1)$$

Assuming $m = 10^{28} \text{ g}$, $A = 10^9 \text{ cm}$, and $Q > 1$, one finds $\tau > 10^{25} \text{ s}$, much greater than the age of the Solar System, $T \approx 10^{17} \text{ s}$. As we argue below, $m \ll 10^{28} \text{ g}$ (at least at present), so that τ is a lower limit. Of course, this argument would not preclude the possibility that an incident body rather than a body in a slowly decaying orbit could have moved towards the Sun and broken up on a much shorter time scale. A cometary or meteoritic supply or an original refractory condensate in the intra-mercurial region is also possible.

We know of no other evolutionary arguments which would suggest either the present position, mass or other properties of a material ring around the Sun. We therefore consider what present dynamical considerations say about any possible ring system.

A ring of particles in orbit about the Sun will give rise to a gravitational quadrupole moment, adding a source of perturbations to the planetary orbits. The most sensitive probe of such a mass quadrupole source is provided by the orbit of Mercury. Two effects are possible. First, the ring could produce an extra contribution to the advance of the perihelion of Mercury's orbit (as well as the other inner planets). The discrepancy between the observed perihelion advance, and the prediction based on newtonian gravity was originally taken as evidence for a planet or ring inside Mercury's orbit⁶. We shall assume here that general relativity is the correct theory of gravity, and take as an upper limit on any possible contribution to the perihelion advance due to the hypothetical ring the uncertainty in the relativistic contribution, that is, $\Delta\phi_{\text{ring}} < 10^{-2} \Delta\phi_{\text{GR}}$, where $\Delta\phi_{\text{GR}}$ is the general relativistic contribution to the perihelion advance, equal to about 43" per century for Mercury⁷. A straightforward calculation shows that a circular ring of particles of total mass M and orbital radius R (having width and thickness much less than R) gives rise to a perihelion advance

$$\Delta\phi_{\text{ring}} = \frac{1}{4} \frac{MR^2 c^2}{GM_{\odot}^2 a(1-e^2)} \Delta\phi_{\text{GR}} \quad (2)$$

where a and e are the semi-major axes and eccentricity of Mercury's orbit, respectively, and $\Delta\phi_{\text{GR}}$ is the expected general relativistic contribution to $\Delta\phi$. (Alternatively, the ring is equivalent to a mass quadrupolar moment $J_2 = [MR^2/2]M_{\odot}R_{\odot}^2$.) With the observed limit on $\Delta\phi_{\text{ring}}$ (or on J_2) we find

$$MR^2 < 4 \times 10^{48} \text{ g cm}^2 \quad (3)$$

Precession of the nodes of Mercury's orbit gives a limit which is about two orders of magnitude higher, assuming that the orbital plane of the ring is the same as the invariant plane of the Solar System.

We next consider the size of any hypothetical bodies. Here one is guided by the various dynamical forces acting on the bodies. The Poynting–Robertson effect drags objects in a circular orbit of radius R into the Sun on a time scale⁸

$$t \approx 3 \times 10^{-20} AR^2 \rho_r \text{ (years)} \quad (4)$$

where $A(\text{cm})$ and $\rho_r(\text{g cm}^{-3})$ are the radius and density of the ring particles. For objects at a few solar radii and densities of a few g cm^{-3} , only kilometre-size objects have survived since the formation of the Solar System. Drag by motion through the solar wind or outer corona are of comparable importance at a few solar radii, negligible at greater distances. We have also examined the so-called Yarkovsky drag on rotating heated bodies⁸ (for all angular rotation rates) and find it places a less restrictive lower limit on the allowed size of the particles.

We now consider constraints on the properties of the ring particles by the requirement of survival against solar irradiation. For most materials, the direct effect of sputtering by the solar particle beams will ablate away only a few centimetres of almost any material per 10^9 years. (Sputtering will, however, considerably darken the surface materials and redden the reflected light, making visual observation less likely—see ref. 9.) The fierce solar radiation field will directly heat the bodies, leading to rapid evaporation. Objects at orbital radius R , with radiation absorption coefficient α will be heated to an assumed uniform surface temperature

$$T = \left(\frac{\alpha R_{\odot}^2}{4R^2} \right)^{1/4} T_{\odot} \quad (5)$$

For a very dark material like carbon with $\alpha \approx 1$, the black-body

equilibrium temperature at $4R_{\odot}$ is $\sim 2,000$ K; for more reflective refractory materials (for example alumina, calcium-aluminium silicate) with $\alpha \approx 0.1$, $T_{\text{eq}} \approx 1,100$ K. To find the vaporisation rate produced at this temperature, we must know the vapour pressure observed or computed for the assumed material composition of the bodies as a function of temperature. No simple analytical expression exists for the vapour pressure as a function of temperature for most materials. Using present numerical data for the vapour pressure as a function of temperature $p = p(T)$, we estimated the vaporisation rate for a variety of materials^{10,11} ($F(T) = p(T) (kT/m_{\text{atom}})^{-1/2} \text{ cm}^{-2} \text{ s}^{-1}$, where m_{atom} as the atomic mass of the vaporising species). Carbon seems to be both abundant and refractory enough to survive in the near solar environment. Graphite seems the likely form. Lewis and Ney¹² have recently examined the conditions for the condensation of refractory grains in circumstellar envelopes and found that for a broad range of C/O ratios, Fe and Fe_3C condense stably and permit graphite formation over a wide range of vapour pressures below 2,000 K.

Combining the minimum size restrictions set by the Poynting–Robertson effect with the vaporisation by solar irradiation, we find that graphite bodies (absorption coefficient $\alpha \approx 1$, density $\rho \approx 2 \text{ g cm}^{-3}$) of radius $A \geq 10$ km could survive both vaporisation and drag for the age of the Solar System, but only at a distance of about $4R_{\odot}$. At a distance of only $2R_{\odot}$, even an object as large as is allowed by considerations of possible tensile strengths ($A \approx 300$ km) would evaporate, no matter what its composition, in less than 10^9 years.

Finally, we look at the possible destruction of the ring by particle collisions. There are two relevant time scales for the collisional evolution of rotating ring particles. First, there is a relatively short time scale for ring flattening and circularisation of orbits. A straightforward analytical calculation gives for this time scale $t_1 = (t_{\text{orbit}}/\pi N)(R/A)^2$, where t_{orbit} is the orbital period at heliocentric distance R and N is the number of ring particles. For $N \approx 10^6$, $R \approx 4R_{\odot}$ and $A \approx 10$ km, $t_{\text{orbit}} \approx 10^5$ s and $t_1 \approx 10^2$ yr. After this time, the only particles surviving collisional breakup or particle accumulation are those in circular orbits. The disrupted particles are irrelevant for the present considerations, and we are only concerned with the remaining particles in circular orbits. Detailed numerical calculations^{14,15}, however, show that the ring subsequently disperses on a much longer time scale $t_2 \approx (t_{\text{orbit}}/3N)(R/A)^4$. For the ring parameters derived above, one has $t_2 \approx 10^{13}$ yr. Thus, an initially present or later formed ring of 10^6 particles in circular orbits will be stable over the age of the Solar System.

We therefore arrive at a consistent picture of the most massive primordial ring allowed to reside around the Sun. It must lie at a distance $R \geq 4R_{\odot}$. Its total mass is, therefore $M \leq 6 \times 10^{25}$ g. It could consist in that orbit of at most $N \leq 10^6$ objects of minimum radius $A \approx 10$ km.

Would such objects be visible optically? If they consisted of graphite or other dark refractory materials with an optical albedo of a few per cent, they might be individually detectable with a visual magnitude of $m_v \approx +8$ to $+10$. Such objects would be observable, in practice, only during a total eclipse of the Sun. Their existence does not yet seem to be ruled out by any direct observations¹⁶.

Finally, note that there is one intriguing piece of observational data which may hint at the existence of such a ring, precisely at the minimal allowable radius we derive above. Several IR observations^{17,18} at $2.2 \mu\text{m}$ and at longer wavelengths find an excess emission peak at $4R_{\odot}$. This peak is not implausibly interpreted as resulting from vaporisation of small silicate grains continually spiralling into the Sun under Poynting–Robertson drag, but we can speculate that the excess may in part be due to a ring of larger bodies in orbit close to the Sun.

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Is SS433 a young binary neutron star?

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The highly variable X-ray and radio star SS433 has received much attention^{1–3} because of its intense emission lines of oscillating frequency^{4,5}. These comparatively narrow lines have been identified as two sets of Balmer and He I lines whose redshifts (and blueshifts) vary sinusoidally, according to

$$\lambda_{\pm}/\lambda_0 = 1.039[1 \pm [0.0765 \cos \omega t + 0.0544]] \quad (1)$$

with a probable period^{5,6} (161.7 ± 0.3) d, as well as a more intense 'stationary' system. Sharp subcomponents of the 'stationary' system vary in redshift⁷ with a period of (13 ± 0.1) d and radial velocity amplitude $K = (76 \pm 7)$ km s⁻¹. This same periodicity probably shows up⁸ in the total optical intensity, with an amplitude of some 0.3 mag. I propose here that the moving lines are the result of stimulated emission from the inner edge of an accretion disk around a rapidly spinning neutron star, pumped by cyclotron radiation. The neutron star is in an eccentric orbit of period 13 d around the Of star SS433, and is forced by its accretion disk to precess with a period of 162 d. The 'stationary' emission line system consists of reprocessed starlight from the outer accretion disk, and the X rays together with light from the two oscillating line systems cool the inner edge of the disk which cuts deeply into the co-rotating magnetosphere of the (inclined) neutron star.

The huge red- and blueshifts of the moving emission lines imply that bulk matter is moving with velocities reaching or exceeding one sixth of the speed of light. They have often been interpreted¹ as coming from two antipodal beams; but there are enormous difficulties in producing a pair of almost relativistic, narrow, monoenergetic, and sufficiently dense beams on planetary-system-scales. If, instead, the lines are emitted by matter orbiting around a compact object², only their narrowness and sine-shaped law of motion are unexpected. But consider a neutron star as the likely⁹ companion of the Of star SS433, whose spin is inclined at an angle χ w.r.t. the normal to the orbit plane and whose magnetic dipole moment is almost at right angles⁹ w.r.t. its spin; once matter is transferred from the primary to the neutron star, at a rate exceeding the Eddington rate, it is likely to form an accretion disk¹⁰ in the orbital plane. This disk will cut into the magnetosphere, in the shape of a thin blade^{11,12}. Magnetospheric braking will slow down its innermost part to slightly sub-keplerian velocities until magnetic support comes weakly into play. The torque T exerted by the asymmetrically confined dipole field on the neutron star has been

calculated by Aly¹¹. Averaged over one revolution period

$$T = 2D^2 \sin 2\chi / 3\pi R^3 \quad (2)$$

where D is the magnetic dipole moment and R the radius of the disk's inner edge, and forces the spin to precess around the normal to the disk plane with a period

$$P_{\text{prec}} = I\Omega / T = 1 \text{ yr } I_{44.5} \Omega_2 R_3^3 B_{12.5}^{-2} / (2 \sin 2\chi) \quad (3)$$

where I is the moment of inertia, Ω the angular velocity, and B the polar surface field strength. The observed period $P_{\text{prec}} = 162$ d requires

$$I_{44.5} \Omega_2 R_3^3 B_{12.5}^{-2} / (2 \sin 2\chi) = 0.44$$

a plausible value, as argued below.

The torque T , when acting on the inner edge of the disk, warps its shape such that orbiting matter acquires a velocity component perpendicular to it which peaks in magnitude where the dipole moment periodically intersects the disk. Radiation emitted at this longitude in near-keplerian motion is seen redshifted, at infinity of a Schwarzschild spacetime, by an amount given by

$$\lambda_{\pm}/\lambda_0 \approx (1 - 3u/2)^{-1/2} \{1 \pm (u/2)^{1/2} (1 - u)^{-1/2} \times [\sin i \cos \omega t + \cos i \, d\theta/d\phi]\} \quad (4)$$

Here $u := r_s/r = 2GM/rc^2$ is the inverse radial distance in units of the Schwarzschild radius r_s , i is the inclination angle, ω the orbital angular velocity, $d\theta/d\phi$ measures the out-of-plane velocity component, and second order effects in u (like the general relativistic light bending effect) have been ignored. A comparison with the observed law of equation (1) yields: $u = 0.05$, $i = 28^\circ$, $d\theta/d\phi = 0.38$.

In comparing equations (1) and (4), I have assumed that the moving emission lines of SS433 come from the two antipodal spots at the inner edge of an accretion disk where it is most warped, so that the spots move with the precession of the neutron star's spin. For a $1.4 M_\odot$ neutron star, the value $u = 0.05$ corresponds to the radial distance $R = 20r_s = 0.8 \times 10^7$ cm which has been anticipated in equation (3). Here the (confined) magnetic field strength is of the order of 10^{10} G (if of the order of 3×10^{12} G at the polar caps).

Can such tiny spots emit more than $3L_\odot$ in the form of Balmer lines? For incoherent radiation, the observed intensities would imply, at temperature T , a radiating area in excess of $(10^{12} T_4^{-1/2} \text{ cm}^2)^2$. Velocity broadening of the lines is $<10\%$ of their velocity shift, hence in the present model the size of the radiating spots must be $<(R/10)^2$ each, and lasing must result in an enhancement by factors of the order of $10^8 T_8^{-1}$. Note, however, that coherent radiation from an antipodal pair of spots at the inner edge of an accretion disk offers plausible explanations for the following observations:

- (1) the two moving line systems often show mirror-image symmetry;
- (2) sharp lines are more easily produced by a coherent mechanism than by an incoherent one, as well as rapid time variability;
- (3) observed deviations from the strict redshift law in equation (1), on the time scale of days, can be understood as being produced by matter sinking more deeply towards the neutron star's surface (and eventually being accreted).

Before discussing the hypothesised laser in more detail, the overall features of this model should be considered. A binary orbital period of 13 d corresponds to a semi-major axis

$$a = (GM/\omega^2)^{1/3} = 5 \times 10^{12} \text{ cm } (M/30M_\odot)^{1/3} \quad (5)$$

$M := M_1 + M_2$, and to orbital velocities

$$v_1 \leq 13 \text{ km s}^{-1} (M/30M_\odot)^{-2/3} \left(\frac{1+\epsilon}{1-\epsilon} \right)^{1/2} \quad (6)$$

$$v_2 \sin i = (M_1/M_2) v_1 \sin i \leq 1.3 \times 10^2 \text{ km s}^{-1}$$

where ϵ = numerical eccentricity, and $\sin i = 0.48$ has been

taken from above. The close separation of $a = 0.3$ AU is consistent with Roche lobe overflow from the primary (whose mass is assumed to be $\sim 28.6M_{\odot}$), and with a strong illumination of the accretion disk. The small velocity amplitude of the primary is consistent with the observational constraint $v_1 < 50 \text{ km s}^{-1}$. An observed $K = 76 \pm 7 \text{ km s}^{-1}$ for the motion of a secondary object is consistent with our $v_2 \sin i \leq 130 \text{ km s}^{-1}$ because the strongest subcomponents will be emitted from the near side of the accretion disk which rotates in a retrograde sense. A variation in the total optical luminosity is explained by a variable binary separation, that is, by a variable illumination of the disk. The disk is probably transparent in the optical continuum but opaque in the UV, IR and optical lines, and retarded cooling would explain an asymmetrical light curve. Note that according to Press *et al.*¹³, a substantial orbital eccentricity is expected to survive for some 10^{10} yr at the calculated separation. In this picture, the disk should also be identified with the large and variable IR source discussed by Giles *et al.*¹⁴. Finally, the relative phases, during the 13-d cycle, of the maxima in redshift⁷ and intensity⁸ can be explained by a substantial angle between the line of apses and the line of nodes.

What is the structure of the (inner edge of the) disk? If confined by a 10^{10} G magnetic field, its internal pressure must be $p = 10^{18.6} \text{ dyn cm}^{-2}$, corresponding to a particle density $n = p/kT \approx 10^{28.6} T_6^{-1} \text{ cm}^{-3}$. This pressure is higher than typical values for accretion disks (see refs 15, 16), probably because a high spin rate of the neutron star hampers accretion so that matter piles up and settles deeper into the magnetosphere. The inner edge of the disk is heated by interaction with the rapidly time-varying magnetic field, in addition to internal dissipation, and cooled by incoherent X-ray emission¹⁷ and coherent line radiation. A total radiation power $L \approx 3 \times 10^{35} \text{ erg s}^{-1}$ emitted near $r = 10^7 \text{ cm}$ implies an effective temperature $T_{\text{eff}} \approx 10^6 \text{ K}$, but X-ray energies $\approx 10 \text{ keV}$ indicate the presence of a much hotter corona, with electron temperatures in excess of 10^8 K . In these conditions (with LTE assumed), the Saha equation yields a (maximum) neutral abundance fraction

$$\gamma = 4 \times 10^{-8} h_{20} T_8^{-3/2} \quad (7)$$

so that the corona is highly ionised. The scale height h is given by

$$h = (kTr^3/GM_*M_p)^{1/2} = 3 \times 10^5 \text{ cm } T_8^{1/2} r_7^{3/2} \quad (8)$$

where M_* is the mass of the neutron star, and M_p the proton mass.

Assuming that the dissipative interaction between disk and rotating magnet leads predominantly to cyclotron radiation, whose power

$$L = N\sigma_T c B^2 / 4\pi = 2 \times 10^5 N B_{10}^2 \text{ erg s}^{-1} \quad (9)$$

should equal the total emission, the presence of $N \approx 10^{30}$ electrons is implied (σ_T = Thomson cross section). This radiation is emitted at frequencies $\nu_0 = eB/2\pi m_0 c = 3 \times 10^{16} \text{ Hz } B_{10}$ and its higher harmonics $\nu_n = (n+1)\nu_0$ caused by a marginally relativistic beaming. The corresponding brightness temperatures

$$T_b \approx 2\pi^2 m_0 c^2 e (\int n ds) (\nu/\Delta\nu) \beta_{\perp}^2 / 3B(n+1)^3 k \\ = 2 \times 10^6 \text{ K} (\int n ds)_{14} (\nu/\Delta\nu)_1 \beta_{\perp}^2 B_{10}^{-1} (n+1)^{-3} \quad (10)$$

exceed 10^6 K up to some $n(\geq 0)$ for almost relativistic electron velocities $v = c\beta$ and a magnetised column density $\int n ds \gg 10^{13} \text{ cm}^{-2}$. This UV radiation floods the inner disk. It ionises the diskal gas, and is partially inverse-Compton scattered to X-ray energies in the hot corona. It is preferentially generated where the disk is most warped, leading to the highest brightness temperatures, to the highest ionisation rates, and to stimulated emission of all the lines of the neutral component. As absorption cross-sections in the lines exceed 10^{-17} cm^2 , the lasing photosphere is extremely thin. Note that the absence of observable

pressure broadening implies $T \geq 10^6 \text{ K } n_{21}^{2/3}$, and that the absence of magnetic signature in the lines (shifts or circular polarisation⁴) implies magnetic field strengths either below 10^6 G or above 10^{10} G . The latter is probably realised. (The former alternative could hold were a non-magnetised photon cushion separating the magnetosphere from the—probably similarly magnetised—central disk.)

Why does SS433 look so different from other binary X-ray sources which involve a neutron star? It may be due to the neutron star's high spin rate, preventing rapid accretion. In the absence of a complete solution¹⁶, one may argue that a low or vanishing accretion rate from a near disk requires a high enough angular velocity of the star, such that the (neutral) force balance radius $r_{\text{fb}} = (GM_*/\Omega^2)^{1/3}$ does not greatly exceed the extent r_{cf} of the closed field line region. For an inclined (static) dipole field and a perfectly conducting infinitely thin 'disk', of inner radius R , the ratio $\xi := r_{\text{cf}}/R$ obeys^{11,12}

$$\xi = [1 + (2tg\chi/\pi \cos \phi)^2]^{1/2}, \quad (11)$$

where χ is the elevation angle of the dipole and ϕ its azimuthal angle. In my model, χ ranges twice from 0° to some maximum angle during one cycle, whence $\xi \geq 1$, and the condition $r_{\text{fb}} \leq r_{\text{cf}}$ implies

$$\Omega \geq [GM_*/(\xi R)^3]^{1/2} \approx 6 \times 10^2 \xi^{-3/2} \text{ s}^{-1} \quad (12)$$

This estimate is unrealistically high, and suggests that the above criterion is too strict in a non-static situation. In any case, the neutron star's spin rate may be very high, comparable with that of the Vela pulsar, and its age correspondingly low, $\leq 10^4$ yr; see ref. 10.

For such a low age, one should still see the supernova remnant that was blown off at the neutron star's birth. An association with the remnant W 50 has been suggested¹⁹.

Finally we should consider the radio structure of SS433. If the secondary involves a rapidly rotating neutron star, there should be an open field line region connecting its polar caps to its wind zone and along which a pulsar wind is blown out. This relativistic wind must intersect the primary's ordinary stellar wind, and give rise to an extended radio structure that shares both the 13-d and the 162-d period. As the pulsar wind will be orientated w.r.t. the neutron star's spin axis, it will illuminate the primary at a variable rate during its 13-d orbital and 162-d precession cycle, thus giving rise to a variable brightness of SS433, as observed⁶.

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Dielectric breakdown by electrically induced chemical decomposition

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Experimental observations and our interpretation of electrical current instabilities in single crystals of ionic metal azides are described here. The subsequent insulation breakdown occurs over wide ranges of fields and delay times. The fields, at room temperature, are of the order MV m^{-1} or lower, and are 2–3 orders of magnitude smaller than the usual dielectric strength found in a homogeneous bulk solid. The breakdown is strongly time dependent and the delay after the application of the field can be up to a few days. We suggest for this low-field long-delay breakdown an ‘electrochemical’ mechanism which seems not to have been envisaged before.

Thoma¹ attributed breakdowns which exhibit extremely long delay times to the growth of conduction filaments due to dislocation structure. Narayan *et al.*² observed that breakdown in MgO single crystals at 1,300 K occurred in a field of 0.1 MV m^{-1} after $>100 \text{ h}$. They proposed that during this incubation period the properties of the materials in the vicinity of the dislocations and small-angle grain boundaries changed. The changes increased the conductance along these pathways, leading to high conductivity filaments, local heating and dielectric breakdown. However, they did not specify the nature of the structural and electrical changes, nor the underlying mechanisms. We have set up a more detailed model for the breakdown in metal azides, but here we will, after summarising the empirical observations, describe only briefly our proposed scheme.

Figure 1 illustrates the behaviour of the current conducted by silver azide; the cases of thallous, lead and sodium azides have been found to be similar. At low fields, the conduction is by mobile interstitial Ag^+ according to previous work on polycrystalline samples^{3,4}. We ascribe the decay of current with time to ionic polarisation at the electrodes, and this is supported by the frequency dependence of a.c. conductivity⁵.

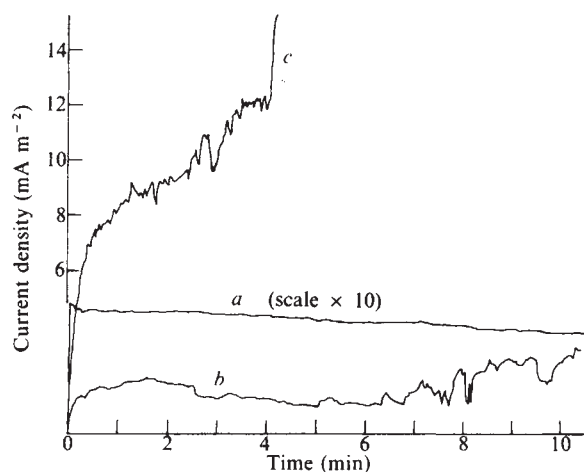


Fig. 1 Current conducted along the *c*-axis of a AgN_3 crystal at a field of *a*, 3.3; *b*, 13 and *c*, 67 kV m^{-1} , in ultrahigh vacuum, and with tantalum pressure-contacted electrodes; qualitatively the same behaviour is observed with silver and graphite electrodes.

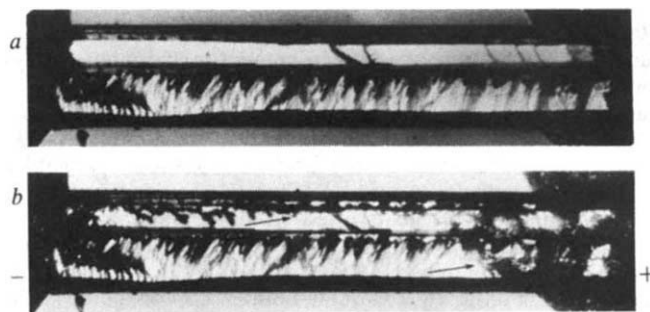


Fig. 2 Photomicrographs of a AgN_3 crystal: *a*, before; and *b*, 5 min after being subjected to 130 kV m^{-1} , arrows indicate new features formed $\times 95$.

At moderate fields $>15 \text{ kV m}^{-1}$ (400, 800 and 200 kV m^{-1} in the cases of TiN_3 , $\text{Pb(N}_3)_2$ and NaN_3 respectively), the current displays large-amplitude non-periodic fluctuations; its average value also increases with time, and at a faster rate when the field is stronger. An estimation of the interfacial fields at the polarised contacts⁵ suggests that the fluctuations are caused by the double injection of electrons and holes into the crystal. We believe that the continuous increase in the current is caused by the formation of metallic filament-like films on the crystal surface; such electrical behaviour has been shown to exist for the case of ultra-thin discontinuous metal films growing on insulating substrates^{6,7}.

In these fields, silver and lead azides have been shown⁸ to give off nitrogen at rates much greater than those corresponding to the electrolysis of the crystals as estimated from their ionic conductivities. Based on a comparison with the mechanism of thermal decomposition of silver azide⁹, we propose the following scheme of elementary steps for the production of surface films. Electrons injected into the conduction band of the insulator constitute a non-equilibrium concentration and so, in their drift towards the anode, a finite number of them may be localised at the electron traps. An interstitial cation may then get discharged at a filled trap, which is thereby reset. On the surface, neutral atoms migrate (see, for example, the case of silver cyanamide¹⁰) to form aggregates so that the discharge is irreversible. The agglomeration gives rise to, first, a network of thin films and, at later stages, clusters of discrete particles on the surface. The appearance of such metal pebbles has been confirmed (Figs 2 and 3); energy dispersive X-ray analyses have shown them to be silver. (See also ref. 9 for transmission electron micrographs of ‘pebbles’ formed by thermal decomposition.)



Fig. 3 Electron micrograph of crystal in Fig. 2*b*: surface features are resolved into ‘pebbles’ $\times 1,350$.

We found that if, during the initial stage of current increase the field was removed, the conductivity returned to its original value. The removal of the field will very quickly stop further generation of silver on the crystal surface, but the accretion of metal atoms in the films at the ambient temperature will continue to change their network structure; the 'pebbles' are permanent, but their number at this stage is small. This means that the number density of the films will continue to decrease with time, and after a sufficiently long time the conductivity will return to the original bulk value. In the accompanying process of field injection of holes, valence electrons are extracted into the anode. The holes drift towards the cathode, some of them combining bimolecularly on the way: $N_3 + N_3 \rightarrow 3N_2$. This process maintains stoichiometry, and leads to the formation and evolution of nitrogen away from the anode¹¹.



Fig. 4 A AgN_3 crystal (1.35 mm long) supporting a corona discharge (arrowed) without dielectric breakdown.

The dielectric breakdown is identified as the current runaway arising from the growth of the metal-like films in number and in size. It is therefore expected to occur after an interval of time that is longer for a lower field, when the production rate of metal atoms is slower, provided the field exceeds the limiting value for the onset of bipolar injection. Indeed we have shown that, for silver azide, the time to breakdown⁵ is <1 s in a d.c. field of 400 kV m^{-1} or greater, increasing to 5 days at 15 kV m^{-1} ; the other three azides show a similar wide range of breakdown times. Also, when the voltage was applied through only one conducting contact no breakdown was observed after many minutes at an estimated field of 300 kV m^{-1} (crystal in air: Fig. 4) or 1 MV m^{-1} (crystal in oil), indicating that the injection of non-equilibrium carriers is necessary. A similar result was obtained in the case of lead azide insulated with Mylar at both contacts¹². Our proposed mechanism predicts that post-breakdown disruptions (due to localised heating by the electronic current in the film network) may start away from either electrode, a fact we have experimentally demonstrated⁵ but which cannot easily be explained in alternative models previously put forward by other authors¹³.

Finally, we suggest that breakdowns of extended delay times may be brought about by metallic conduction caused by certain 'electrical decomposition' processes in some ionic metal compounds besides the azides, such as halides, iodides, oxides and hydrides.

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Abnormal characteristics in crystallisation of $Ca(PO_3)_2$ glass

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Although glass is known not to crystallise below the glass transition temperature (T_g), some block specimens of metaphosphate glasses such as $Ca(PO_3)_2$, $Sr(PO_3)_2$ and $Ba(PO_3)_2$ have been found to crystallise easily at temperatures considerably lower than T_g ; however, thin films of the glasses do not exhibit this behaviour^{1–3}. The intrinsic characteristics of $Ca(PO_3)_2$ considered here suggest that under stress the rate of crystal growth depends on thickness, and that long polycrystalline fibres may be produced in which the crystals are orientated towards the direction of elongation.

When the chemical composition of a glass is the same as that of the growing crystal, equation (1) is applicable for most glasses⁴,

$$u\eta/\Delta T \approx 0.1 \quad (1)$$

where u is crystal growth rate (cm s^{-1}), η is viscosity (P), and ΔT is the degree of undercooling. In $Ca(PO_3)_2$ glass, the crystalline phase has the same chemical composition as that of the glass. Hence, u is calculated from equation (1) to be $\approx 10^{-7} \text{ } \mu\text{m min}^{-1}$ or several micrometres per 100 yr at T_g (melting point of $\beta\text{-Ca(PO}_3)_2 = 980^\circ\text{C}$, $T_g \approx 500^\circ\text{C}$, $\eta \approx 10^{14} \text{ P}$). This calculated rate of crystal growth is almost the same as that obtained by extrapolating the experimental data¹ for the thin film at temperatures higher than T_g . Thus, the crystal growth rate (u) of the thin film holds for equation (1), showing the usual crystallisation mechanism. However, u of the block specimen of $Ca(PO_3)_2$ glass is extraordinarily large reaching 10^7 – 10^8 times that of the thin film at temperatures near T_g , thus showing an unusual crystallisation mechanism. This abnormally high rate of

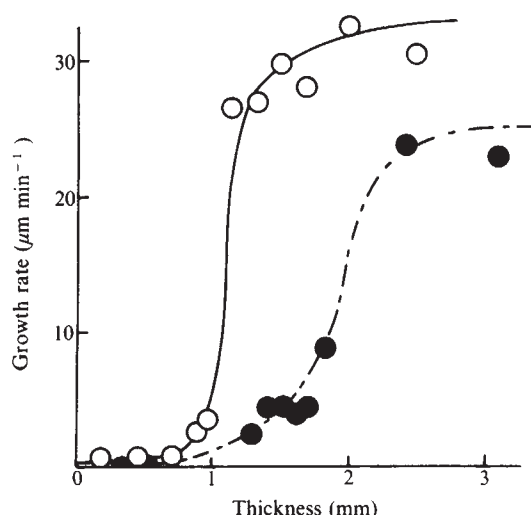


Fig. 1 Dependence of the crystal growth rate on the thickness of the $Ca(PO_3)_2$ glass plate specimen. ○, 560°C ; ●, 540°C .

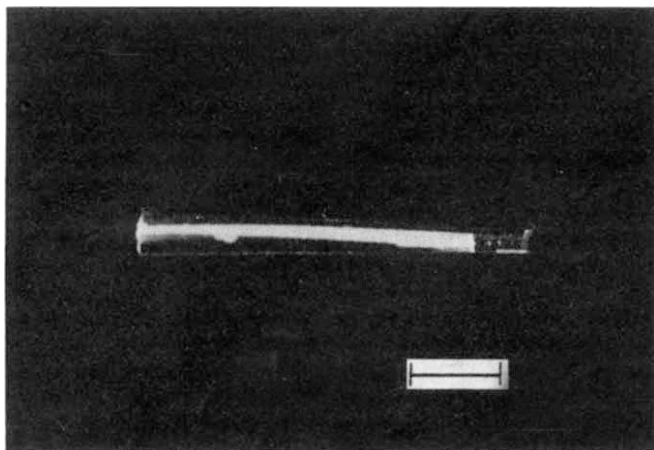


Fig. 2 Photo of orientated crystallisation in an elongated $\text{Ca}(\text{PO}_3)_2$ glass rod. Elongation, 300% at 540 °C; Heat treatment for the crystallisation, 510 °C for 6 h. Scale bar, 5 mm.

crystal growth for block specimen of $\text{Ca}(\text{PO}_3)_2$ glass has been explained in terms of induced tension in the molecular chains at the glass–crystal interface, where tension is induced by a volume difference between the crystalline and glassy phases¹.

These results suggest that the crystal growth rate depends on the shape or thickness of the glass specimen. Figure 1 shows the dependence of the rate of hemispherulitic crystal growth on the thickness of $\text{Ca}(\text{PO}_3)_2$ glass plate specimen. Abrupt change in the growth rate occurs at ≈ 1 mm thickness at 560 °C and ≈ 2 mm thickness at 540 °C, respectively, the critical thickness at which these abrupt changes take place becoming larger as the temperature decreases. I believe that this critical thickness is determined by competition between the rate of relaxation of the induced tension in the molecular chains at a growing crystal–glass interface and the rate of the crystal growth (assumed to be without stress relaxation) at a given temperature.

Another characteristic crystallisation property for $\text{Ca}(\text{PO}_3)_2$ glass is crystallisation orientated towards the direction of tension. A fine glass rod (3–4 mm in diameter) was produced from a $\text{Ca}(\text{PO}_3)_2$ glass rod held at 540 °C (a little higher than T_g) by pulling and quenching under a force of 8 kg cm^{-2} to become three times the length of the original rod. This specimen was heated at 510 °C for 6 h under no load, resulting in orientated crystallisation towards the direction of elongation of the specimen; note that although the crystallisation occurred preferentially inside the glass rod, the surface of the specimen remained uncrystallised (Fig. 2). When not elongated, hemispherulite only usually grows at arbitrary places of the glass surface^{1,2}; therefore orientated crystallisation must be due to stretching of the glass specimen which results in orientation of molecular chains in the glass towards the elongation direction. At temperatures around T_g , although the viscosity is low enough to allow the glass to be stretched by a sufficiently large tensile stress, the mobility of the molecular chains or chain segments is not high enough for the orientation produced by stretching to be lost during the thermal treatment to which the glass is subjected. The crystal growth rate towards the elongation direction is very high, reaching at least 10 times the growth rate of hemispherulite—usually grown from the block specimen of the glass prepared with no applied stress, the growth rate depending on conditions for the preparation of the fine rod (elongation times, elongation temperatures, rate of quenching).

It is surprising that $\text{Ca}(\text{PO}_3)_2$ glass exhibits intrinsic crystallisation below T_g , that the crystal growth rate depends on the thickness, and that the crystallisation may be orientated under tension. These phenomena have not yet been found in inorganic glasses. I believe that these crystallisation characteristics are due to structural features in that $\text{Ca}(\text{PO}_3)_2$ is comprised of molecular chains in both the glass and crystal. We can expect unusual

behaviour in this type of glass that has never been observed in a glass network structure such as commercial industrial glasses.

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¹³C/¹²C and ³⁴S/³²S ratios in magmatic gases from ridge volcanism in Afar

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Most data on the stable isotope composition of deep-seated carbon and sulphur come from igneous rocks and low-temperature gases. Very little information comes from high-temperature gases which escape from magmas ($T = 1,000 \pm 200$ °C), even though these are the best specimens of primary igneous fluids. Moreover, the only available ¹³C/¹²C data on carbon in high-temperature basaltic gases^{2–4} are largely divergent: low ratios from –14 to –26‰ versus PDB were measured in gases from Hawaii^{2,3,5}, which is consistent with the values for reduced carbon in basalts⁶, whereas higher ratios around –6.5‰, typical of carbon in most of carbonatites^{7,8}, diamonds⁹ and kimberlites¹⁰, have been found in gases from northern Afar⁴ (P.A. and M. Javoy, in preparation). The high-temperature gas samples collected from the November 1978 Asal rift eruption, southern Afar¹, have provided new isotope data on basaltic igneous volatiles, released from upper mantle magmatism¹.

Four gas samples were collected from the fissure which generated the eruption ($T = 1,070$ °C) and another four samples were taken from outpoured lava flows, 60 m away from the fissure ($T = 800$ °C)¹.

Chemical compositions were determined by gas chromatography and are reported in Table 1 together with the isotopic results. For isotopic measurements, CO_2 and SO_2 were separated from the mixtures by differential freezing in a high vacuum Pyrex line: after the non-condensable gases in liquid nitrogen were pumped (CO , H_2 , COS , N_2 , O_2 and Ar), melting dimethylbutane (-140 °C) and dry ice acetone (-80 °C) traps were used to isolate CO_2 from SO_2 , and SO_2 from residual water vapour, respectively. Complete extraction was controlled with a precision pressure gauge, and the proportions of gas recovered agreed with the chromatographic results ($\pm 2\%$). Isotopic analyses were performed on a double-collecting micromass spectrometer. Results are expressed in the usual δ notation (1) (ref. 12). Standards are PDB for carbon, SMOW for oxygen and Canyon Diablo Troilite for sulphur. The accuracy of the mass spectrometric analyses (1σ) is better than 0.1‰ for carbon and oxygen, 0.3‰ for sulphur.

$$\delta = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 1,000, \quad (1)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{34}\text{S}/{}^{32}\text{S}$.

Although the samples are largely air contaminated, as shown by their high N_2 contents and $\text{O}_2 + \text{Ar}/\text{N}_2$ ratios which are similar to the atmospheric ratio (0.280), the corresponding CO_2 air contribution amounts to <1% of the total CO_2 content of the samples, and is only significant in the 800 °C samples, particularly in sample no. 13. Assuming that ~ 0.03 mol% of atmospheric CO_2 with a $\delta^{13}\text{C} \approx -7\%$ were added to those samples which contain about 78 mol% of N_2 , we can restore the $\delta^{13}\text{C}$ of true volcanic CO_2 : corrected values are in parentheses in Table

Table 1 Chemical and isotopic compositions of gases from the Asal eruption

Sample:	Erupting fissure (1 or 2) (1,070 °C)				Lava flows (800 °C)			
	1 A92	1 A89	2 A88	2 G82	no. 6	no. 13	no. 5	no. F3
H ₂ O (mol%)	79 ± 3	79 ± 7	ND	77 ± 7	ND	ND	ND	ND
Dry volcanic gases (mol%)	21	21	—	23	—	—	—	—
CO ₂	18.9	18.7	15.9	14.2	90.7	99.9	94.4	93.2
SO ₂ (mol%)	80.4	80.8	84.0	84.9	9.3	0.1	5.6	6.8
H ₂ S of dry	0.0004	0.02	0	0.01	0	0	0	0
H ₂ volcanic	0.25	0.06	0.05	0.22	0	0	0	0
CO gases	0.35	0.30	0.22	0.59	0	0	0	0
COS	2×10^{-4} – 10^{-3}							
C/S	0.24	0.23	0.19	0.18	9.7	10 ³	17	14
N ₂ (mol% of dry gases in samples)	45.0	50.9	47.4	57.9	81.0	78.6	80.3	80.4
O ₂ + Ar/N ₂	0.244	0.251	0.236	0.266	0.180	0.273	0.178	0.188
δ ¹³ C ± 0.1‰ vs PDB(CO ₂)	−5.9	−5.3	+0.2	−4.8	+2.3 (+2.4)	+1.5 (+2.2)	+1.9 (+2.0)	+2.1 (+2.2)
δ ¹⁸ O ± 0.1‰ vs SMOW(CO ₂)	+8.2	+7.8	+8.6	+8.2	+16.0	+20.8	+16.1	+15.9
δ ³⁴ S ± 0.3‰ vs CD(SO ₂)	−3.1	−2.9	−2.3	−2.8	−0.1	ND	−4.8	−0.2

ND, not determined; 0 means under the limits of detection. All the samples were collected in previously evacuated Pyrex bottles containing P₂O₅ desiccator in excess to trap water vapour. A silica collection pipe was used to lead the gases into the bottles¹.

1. Except for sample 13, corrections are within the accuracy possible for mass spectrometric analyses and allow a reliable determination of the ¹³C/¹²C ratio in volcanic CO₂.

CO₂ accounts for nearly the whole carbon content of the gases, as CO and COS when detected are only minor components. Note that both reverse C/S atomic ratios and opposite-sign δ¹³C values distinguish the 800 °C samples (C/S > 10, δ¹³C > 0) from 1,070 °C samples (C/S = 0.2, δ¹³C < 0). Such a discrepancy, and such a high δ¹³C (+2.2‰ versus PDB) in the gases from lava flows, were surprising: the exhibited value fits in with the isotopic range for ¹³C-rich marine limestones⁶.

This might be related to the presence, near to the eruption point, of lacustrine carbonates deposited from the Asal lake basin (155 m below sea level) since this one was isolated from the marine gulf of Ghoubbet-Tadjurah and extended over a wider area of the Asal graben's floor than it is at present¹³. Carbonates were collected in the field near to the sampling sites for chemical and isotopic composition analysis (Table 2).

The results (δ¹³C = +0.85‰) show that a thermal decomposition of such a magnesium-rich calcite could produce CO₂ with a δ¹³C similar to that found in the 800 °C samples: if we assume that isotope equilibrium was maintained between the carbonate and the CO₂ released, and that the fractionation factor for calcite can be used, then a 1.4‰ ¹³C enrichment in CO₂ would correspond to an equilibrium temperature of about 800 °C (ref. 14). Isotopic equilibrium conditions may have been maintained by the thickness of the lava field (10m) that allowed the gases to escape only from a few points where the upper lava crust was broken. Thus, carbonate sediments, partly overlapped by the lava flows, seem the most probable source for the CO₂ sampled outside the fissure.

δ¹³C of sample A88 (+0.2‰), collected from one of the two vents we used above the erupting fissure, suggests a local mixing between a light magmatic CO₂ and the carbonate-derived CO₂. If we consider the δ¹³C value from sample A92 (≈ −6‰) the most representative of unmixed carbon dioxide, we may evaluate the possible contributions from carbonate-derived CO₂ to the other three samples: 9%, 76% and 15% for samples A89, A88 and G89, respectively. It is possible that superficial CO₂ may also have made a (small) contribution to sample A92.

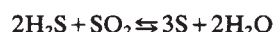
This −6‰ value for uncontaminated CO₂ from the fissure is very similar to that of magmatic CO₂ from the Erta Ale lava lake, on the northernmost spreading axis in Afar (−6.4 ± 0.4‰)⁴. It could also be compared with the δ¹³C of CO₂ trapped in fresh tholeiitic basalts from the mid-Atlantic (−7.6 ± 0.5‰)¹⁵ and east Pacific (−5.4 to −5.8‰)¹⁶ sub-oceanic ridges. Then, it falls within the isotopic range for most of carbonatites, diamonds and

kimberlites in which carbon is usually considered to derive from a deep-seated source.

The shallowness of the mantle beneath the Asal-Ghoubbet ridge¹¹, the thinning of the oceanic crust beneath the axis indicated by the seismic profiles¹⁷, the lack of continental-type crust, the axial situation of the eruption point¹, the high temperature of the gases and their close relationship to the magma feeding channel, suggested that the mantle would be a probable source of the CO₂ released from the fissure. The consistency of the δ¹³C obtained with that of most of presumed deep-seated carbon-rich reservoirs supports such an origin. The close similarity of the ¹³C/¹²C ratios in Asal and Erta Ale magmatic gases indicates that the mantle beneath Afar's spreading axis would have a uniform carbon isotope composition, comparable with that of carbon from other sub-oceanic ridges^{15–16}.

The much lower values measured in magmatic gases from Hawaii^{2,3,5} suggest either (1) lighter ¹³C/¹²C ratios in Hawaiian magma sources, the carbon in plumes from deep mantle being relatively ¹³C-depleted compared with carbon in shallow mantle rises beneath oceanic ridges; or (2) only local contributions as some of the gases were collected from lavas having flooded over organic material². The difference between Asal samples from the fissure and outside the fissure emphasises that lava flows are inadequate sampling sites, being easily contaminated by environmental fluids.

SO₂ constitutes the whole sulphur content of the gases, as H₂S and COS are trace components. Thermodynamical equilibrium in collection conditions (1 atm, 1,070 °C and $p_{O_2} = 10^{-9.4}$ atm)¹ would lead to higher H₂S/SO₂ ratios than are found in the samples (8×10^{-3} compared with 10^{-4}). The lower O₂ + Ar/N₂ ratios in samples than in air indicate that high-temperature air oxidation would be responsible in the reduction of the H₂S proportions. Residual traces of H₂S could also have later reacted with SO₂ in the sampling bottles, according to the reaction



which brings about a relative ³⁴S enrichment in SO₂. But the lack of sulphur precipitation in the bottles during the few days that they were waiting for analysis suggests that small proportions of H₂S compared with SO₂ made this reaction inefficient. (Water vapour, which favours the reaction, was trapped on P₂O₅ desiccator.) Hence, the δ³⁴S of SO₂ would account for the original δ³⁴S of total sulphur in the gases. δ³⁴S in 1,070 °C samples are homogeneous, around a mean value of −2.8‰ versus CD standard; those in 800 °C samples are less homogeneous, but they enclose the former value.

Table 2 Chemical and isotopic compositions of lacustrine carbonates from Asal rift

	(wt%)	$\delta^{13}\text{C}$ vs PDB (‰ ± 0.1)	$\delta^{18}\text{O}$ vs SMOW (‰ ± 0.1)
CaCO_3	55%		
MgCO_3	28%	+0.82	+44.2
Amorphous silica	17%	+0.88	+44.6

Chemical analyses were carried out using an X-ray energy dispersion spectrometer. For isotopic analyses, 1 mg of carbonate was reacted with 100% H_3PO_4 acid at 50 °C for 12 h, after heating 390 °C for 45 min.

This -2.8% $\delta^{34}\text{S}$ is low in the classical range for igneous sulphur in basaltic rocks^{18–20} and fumarole gases²¹, the average composition of which usually lies close to the meteoritic standard value. Comparison with the $\delta^{34}\text{S}$ of SO_2 from Erta Ale, $+1.0 \pm 0.3\%$ (P.A. and M. Javoy, unpublished data), suggests that sulphur in Asal gases originated from a slightly ^{34}S -depleted source, unless continuous loss of SO_2 involved a progressive ^{34}S depletion in the residual sulphur which escaped from the magma, as the feeding intrusion seems to have been a rather closed system¹ and our samples were collected during the later degassing trend of the eruption. If we assume that the initial $\delta^{34}\text{S}$ of total sulphur in the magma was near zero, that about 90% of the original sulphur content had already escaped at the collection time and that a Rayleigh-type isotope distillation took place between SO_2 and the magmatic sulphides, then a fractionation factor of 1.00122 is obtained; this is compatible with the value extrapolated from Sakai's data for the $\text{SO}_2/\text{S}^{2-}$ equilibrium fractionation at temperatures of about 1,200 °C (ref. 22). Otherwise, kinetic isotope fractionation would have concentrated ^{34}S in the residual sulphur and led to a higher ratio than is observed.

This relatively low $\delta^{34}\text{S}$ excludes superficial contributions to the volcanic fluids from sulphates in the marine waters which circulate into the graben from the Ghoubbet to Asal lake ($3,900 \text{ mg l}^{-1}$)²³ and in the saline waters of the lake itself ($4,300 \text{ mg l}^{-1}$) which is 2 km away from the eruption point: the SO_2 produced by high-temperature reduction of such sulphates ($\delta^{34}\text{S} = +20\%$)²³ would actually be ^{34}S -rich compared with SO_2 in the gases, isotope fractionation between SO_4^{2-} and SO_2 remaining close to zero at temperatures above 600 °C (ref. 22). A similar lack of chloride contributed by the environment was previously reported from chemical analyses of gases and related sublimates¹.

Thus, carbon and sulphur outgassed from the fracture which generated the recent Asal eruption derived from a probable mantle source because of both the exceptional tectonic setting of the Asal rift and their stable isotope composition which was similar to that of comparable deep-seated material.

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The Lizard complex as an ophiolite

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The Lizard complex of south Cornwall consists of an assemblage of peridotites, gabbros, hornblende schists, metasediments, dolerites and metadolerites and acid-basic banded gneisses (the Kennack gneisses). It is bordered to the north by a zone of chaotic sediments with phacoidal inclusions of pillow lavas, greywackes and quartzites (the so-called Meneage crush zone). This zone can be traced eastwards into the Roseland district of south Cornwall¹. The age of the complex is uncertain. Dates (largely K–Ar) obtained from the Kennack Gneiss and the majority of hornblende schists are mainly between 370 and 390 Myr, although Green^{2,3} considered the peridotite to be older than the oldest date obtained from the hornblende schists, at 492 Myr. Metadolerite dykes have recently⁴ been given ages of approximately 400 Myr. All the above are thought to represent minimum ages due to the possibility of argon loss during subsequent uplift, cooling and mild metamorphism. Since the work of Green³, the Lizard complex has been regarded by many as the type example of a hot diapiric mantle intrusion into continental crust. Intrusion is thought to have occurred during a period of regional metamorphism and to have resulted in the imposition of a contact dynamothermal aureole on the hornblende schists at the margin of the peridotite body. The gabbro is regarded as a later and unrelated intrusion, and the Kennack gneisses were attributed to intrusion of acid magma along sheared basic dykes in peridotite. Thayer⁵ suggested that the complex was ophiolitic, basing his views on the field relationships of gabbros and peridotites which indicated that the two lithologies might be temporally and genetically related. General comparisons have since been made between the Lizard complex and the well-documented ophiolite sequences^{6–10}, although little detail has generally been given. I provide here relevant details which suggest that the Lizard complex is ophiolitic in origin.

The east coast section of the Lizard complex is thought to form a rock association distinct from the remainder of the complex and to be separated by a major fault (Fig. 1a). There are four east–west trending divisions of the east coast section, which from north to south are: a dyke complex in gabbro; massive, variable and layered gabbro; troctolites and related rocks in peridotite; and peridotite.

The dyke complex consists of subequal amounts of variable gabbros and NW–SE trending subvertical tholeiitic metadolerite dykes. Small amounts of hornblende leucodiorite form segregations in the gabbro and have locally intruded fractured metadolerite dykes forming net-veined complexes. The base of the dyke complex is at Godrevy Cove where there is a rapid transition southwards into gabbro containing only a small percentage of dykes. Subvertical, E–W trending cumulate layering produced by varying proportions of olivine, plagioclase and occasionally clinopyroxene is locally preserved; rare mineralogically graded units 'young' to the north. The present orientation of the cumulates demonstrates that the whole sequence has been rotated through a large angle downwards to the north about an east–west axis. These cumulates are thought to have been deposited on a subhorizontal or gently dipping surface on a floor of peridotite, although subsequent gabbro intrusion and high temperature deformation have obscured many of the original relationships. The remaining gabbro has less well-defined mineralogical variations and is variable between strongly laminated (mostly the plagioclase-rich varieties) and non-laminated. The contact with peridotite at

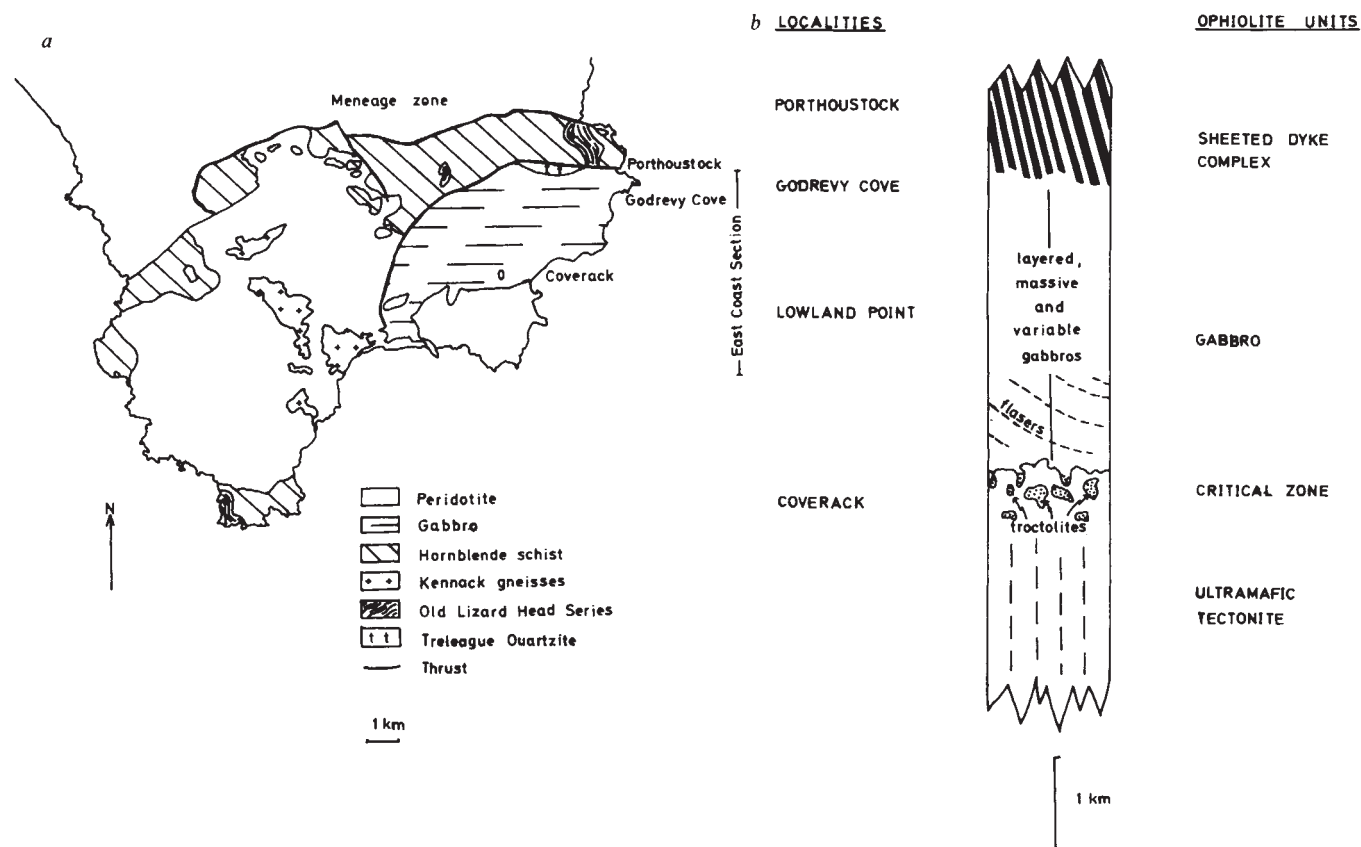


Fig. 1 a, Simplified geological map of the Lizard complex. b, Schematic section of the east coast section and a comparison with an ophiolite succession.

Coverack in the south is a broad zone in which olivine-rich gabbroic and ultramafic rocks ('troctolites') ranging from dunites to olivine gabbros, are complexly interdigitated with peridotite. The troctolites are considered to be the first (and therefore olivine-rich) cumulates which separated from the gabbroic magma, which overlay the peridotite and which were subsequently deformed and partially remobilised, resulting in the inclusion of blocks of peridotite. A possibly slow rate of removal of melt from the peridotite may have resulted in the precipitation of troctolites in pods within the peridotite. Partial-melt textures in the host peridotite are encountered locally in this zone. Rudely-banded lherzolites and harzburgites containing little or no troctolite outcrop further to the south.

The similarity in both the lithologies present and the order in which they occur to the rock succession encountered in an ophiolite sequence (Fig. 1b) is striking and considered to be sufficient evidence to accept this as a truncated ophiolite sequence. This interpretation is in complete contrast to that of Green³ who considered the gabbro to form part of an *in situ* ring intrusion into peridotite.

Of prime importance to the proposed ophiolitic nature of the complex is the relationship between peridotite and gabbro; in ophiolites these are thought to have a parent-daughter relationship. Gabbro in the Lizard complex occurs only within peridotite and not in other lithologies—fortuitous if these two lithologies are not related. Examination of Green's lithological map and the author's field observations show that the gabbro is further restricted to the primary-type peridotite and does not occur in the recrystallised varieties except as isolated and deformed scraps. This implies that the association of peridotite and gabbro existed before their juxtapositioning with the surrounding hornblende schists (the event in which some of the peridotite was recrystallised), a feature common to many other ophiolites. Partial-melt textures at Coverack and locally elsewhere involve gabbroic veins with gradational boundaries against the surrounding peridotite. These support a genetic relationship, which is supported further by the relative rare

earth contents of the two lithologies¹¹. Thus the proposal for the ophiolitic nature of the complex is strengthened.

The remainder of the Lizard complex, which contains very little gabbro is considered to be the peridotite component of the ophiolite sequence together with the underlying metabasic and metasedimentary sub-ophiolitic rocks (the hornblende schists, the Old Lizard Head series rocks and the Kennack gneisses). These lithologies will be reported elsewhere but the salient points are noted here. Two varieties of hornblende schist were distinguished by Flett¹² and subsequently by Green³—the Traboe and the Landewednack hornblende schists. The Traboe variety occurs near the peridotite and was considered to be the upper amphibolite and granulite facies derivative of the amphibolite-grade Landewednack variety, the agent of metamorphism being the intrusive peridotite. Chemical data, however¹³, show that the two groups are chemically distinct and cannot therefore be metamorphic equivalents. The Traboe schists are chemically more 'primitive' than the Landewednack schists. Both the chemical and field evidence support derivation of the Traboe schists from dominantly intrusive and cumulate gabbroic rocks and the Landewednack schists from dominantly intrusive and extrusive basaltic rocks. All are tholeiitic and those which were derived from basaltic protoliths have an oceanic chemistry (Fig. 2). It is proposed that the hornblende schists were derived by deformation of an ophiolite sequence adjacent to and chemically slightly different from that of the east coast section. The transition from the Landewednack variety to the Traboe variety reflects the original transition from the extrusive basaltic to the cumulate gabbroic portion of the ophiolite. True prograde metamorphic sequences are thought to be restricted to those schists below the peridotite from Kennack southwards to Landewednack. Much of Green's prograde metamorphic sequence is thought to be a feature of retrogression.

The Kennack gneisses show abundant evidence of formation by an *in situ* segregation process, in contrast to the artieritic origin proposed by Green, although this is undoubtedly locally true. Detailed petrographic evidence together with the

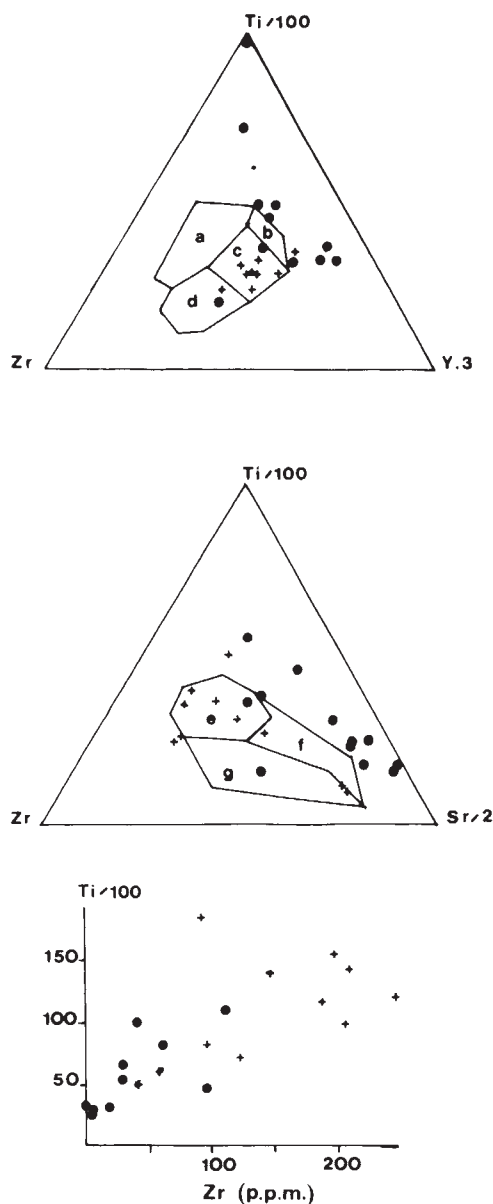


Fig. 2 Geochemistry of the hornblende schists. +, Landwednack; ●, Traboe. Field divisions from Pearce and Cann²⁸: a, within place basalts; b, c, low potassium tholeiites; c, ocean floor basalts; c, d, calc alkaline basalts; e, ocean floor basalts; f, island arc basalts; g, Calc alkaline basalts. The large scatter of data points of the Traboe is a direct result of their low incompatible element contents resulting from their probable derivation from cumulative gabbros.

chemistry of the acid and basic portions suggest that the most likely process was one of partial anatexis of sediments and volcanics. The spatial restriction of the gneisses and the parallelism of the banding to the margin (base) of the peridotite suggests that this was achieved during overriding of the hot peridotite body. The deformation during assembly of the Lizard complex is thought to have resulted in the recrystallisation of the margins of the peridotite to lower pressure assemblages (the recrystallised anhydrous and hydrous varieties of Green³).

The question remains, however, as to whether this ophiolite is a fragment of oceanic crust, as is commonly proposed for many other ophiolites¹⁴⁻¹⁷. The complete absence of rocks of continental affinities within the east coast section is thought to be strong evidence in support of an oceanic origin for the complex. Deformation of the gabbroic cumulates occurred throughout their cooling history and ranged from slumping of unconsoli-

dated cumulates through crystal-mush deformation in a direction subparallel to the layering to the formation of flaser gabbros and finally to brittle faulting. Flaser gabbro zones are concentrated in the south of the gabbro in the lower part of the original cumulate pile and represent low-angled slides developed under amphibolite-facies conditions during cooling of the gabbro. The above, combined with the large degree of crustal extension indicated by the dykes of the dyke complex, demonstrate that crystallisation of the complex occurred in a dominantly tensional and tectonically unstable environment. This is certainly consistent with an origin of the complex at a constructive plate margin. Chemical analyses of the basaltic dykes (ref. 13 and G.A.K., in preparation) indicate a close similarity in both major and trace-element compositions to oceanic tholeiites (Fig. 3). The gabbroic cumulates have tholeiitic affinities and are also thought to have been derived from an oceanic tholeiite magma. Geochemical evidence therefore also supports an interpretation of the Lizard ophiolite as a fragment of oceanic crust. If present models for the generation of oceanic crust are correct¹⁸⁻²⁰, then the gabbroic cumulates and dykes should be derived from the same primary magma. The geochemistry of the dykes at Porthoustock and the gabbroic cumulates of the Lizard broadly support this proposal (Fig. 4), although these results are at variance with those of Floyd *et al.*²¹.

The extrusive portion of the ophiolite sequence is absent south of the Lizard boundary thrust. However, pillow lavas occur within the Meneage crush zone, which is believed to be a

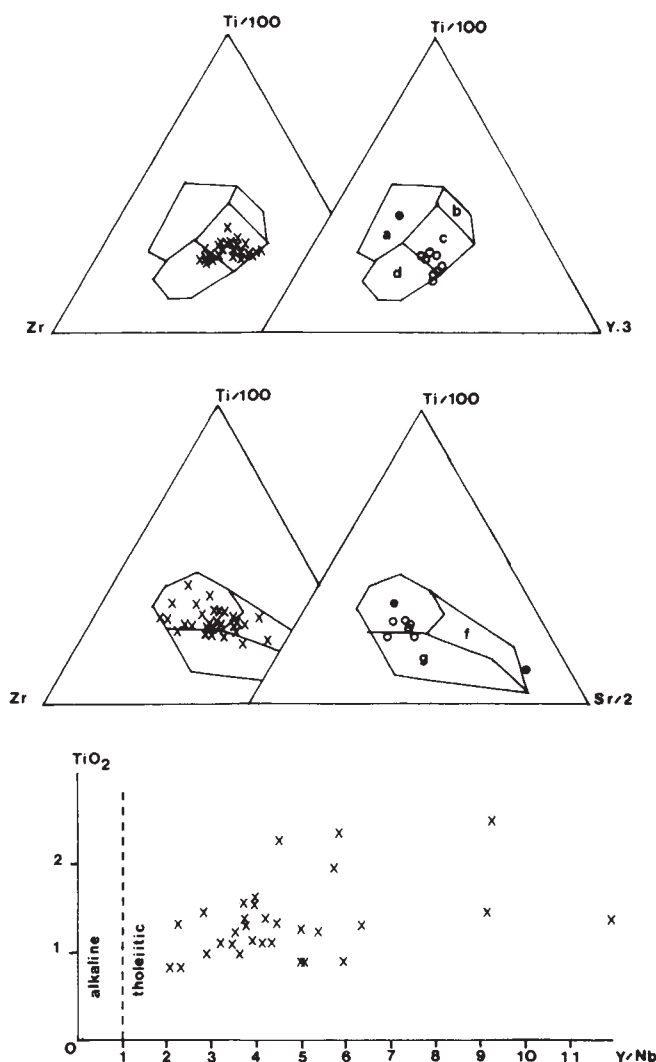


Fig. 3 Geochemistry of basaltic dykes (x) and lavas (○). ●, Average SW England tholeiite. Fields as for Fig. 2. Alkaline-tholeiite discriminant diagram from Floyd and Winchester²⁹.

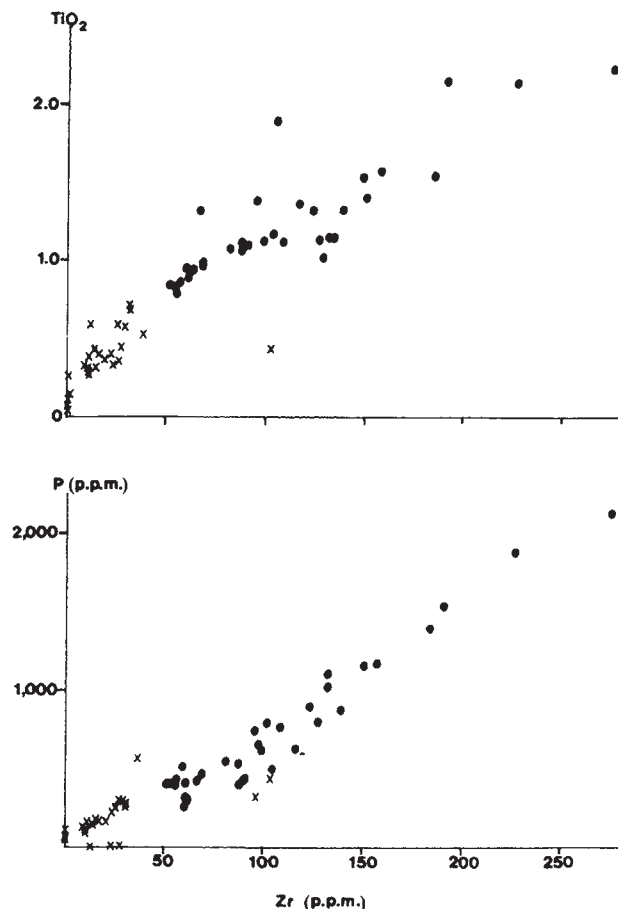


Fig. 4 Interrelationship of trace-element contents of dykes (●) and cumulate gabbros (×). Approximately straight-line trend with gabbros plotting at low concentrations supports a cogenetic relationship.

melange produced beneath and in front of the Lizard ophiolite during its emplacement. The chemistry of the contained lavas is closely allied to rocks of the Lizard complex (Fig. 3) and quite different from the remaining greenstones of South-west England^{1,13,22}. It is therefore thought possible that these lavas are part of the extrusive portion of the Lizard ophiolite or the surrounding oceanic crust which was incorporated into an olistostrome ahead of the ophiolite during its emplacement, and which were subsequently overridden.

The acceptance of the Lizard complex as obducted oceanic crust has important implications for the geological history of SW England. Thus models such as that of Floyd²³ in which SW England was never underlain by oceanic crust are thought to be, in part at least, incorrect. However, I would support Floyd's interpretation of the geochemistry of other SW England greenstones in suggesting that the ocean crust did not extend anywhere north of the Lizard boundary thrust or its extension eastwards. Equally, therefore, the models of Johnson²⁴, Burrett²⁵, Laurent²⁶ and Burne²⁷ are thought to be incorrect in suggesting that all of SW England was underlain by oceanic crust. A knowledge of the age of generation and emplacement of this oceanic crust is important in the development of geotectonic models for this region. It is thought that the ages of up to 403 Myr (late Silurian) obtained from the dykes of the dyke complex⁴ most closely approximate the age of generation. Dates obtained from the gabbro would also be definitive (work on this is in progress). The concentration of ages determined from the hornblende schists and the Kennack gneisses (370–390 Myr)² are thought to date the assembly and beginning of emplacement of the ophiolite. The short time between the generation and emplacement of the complex is a feature common to many ophiolites.

In conclusion it is thought that many features of the Lizard complex do not support an origin by diapiric intrusion of peridotite into continental crust. The geological and geochemical features are consistent with formation as part of late Silurian (?) oceanic crust followed by emplacement of the resulting ophiolite sequence over the continental margin of SW England in early Devonian times.

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High sediment yields from major rivers of the Western Southern Alps, New Zealand

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Suspended sediment yields have been determined by combining suspended sediment rating relationships with water flow records for gauged rivers of South Island, New Zealand. Rivers draining the Western Southern Alps have specific annual yields about 10 times higher than world average rates for mountainous areas. Their short steep catchments, rising in elevation from near sea level to over 3,000 m, support dense unmodified podocarp hardwood and beech forests below 1,000 m. The drainage basins are virtually undisturbed. A simple positive power law relationship between specific annual suspended sediment yields and the extremely high mean annual rainfalls explains almost all the variance in yield for catchments of the western region. This simple law may also hold for undisturbed catchments having a temperate maritime climate, in other areas of the world.

Suspended sediment yields (Table 1) were calculated by integrating suspended sediment rating relationships with continuous flow records of the rivers. These rating relationships are of the form

$$C = aQ^b$$

where C is the suspended sediment concentration in kg m^{-3} and Q is the water discharge in $\text{m}^3 \text{s}^{-1}$. Exponents are similar for all major rivers ($b = 2.2$ to 2.7); coefficients vary through several orders of magnitude ($a = 1.80 \times 10^{-3}$ to 1.87×10^{-5}) partly reflecting the wide variation in catchment areas. The range of suspended sediment concentration values, displayed in Fig. 2 for two typical rivers, is not exceptional. Stage discharge ratings on the unstable channels are defined by a regular gauging

Table 1 Mean annual specific suspended sediment yields and other relevant data for gauged East Coast major rivers and for all gauged West Coast rivers of South Island, New Zealand

	Catchment area (above gauging station) (km ²)	Mean annual specific suspended sediment yield (tonnes km ⁻² yr ⁻¹)	Mean annual rainfall (mm)	Mean annual flow (m ³ s ⁻¹)	Period of sediment record (yr)
East Coast					
Wairau	3,430	870	1,600	114	17
Waiau	1,980	1,300	2,000	90	10
Waimakariri	3,210	1,670	1,900	120	5
Rakaia	2,640	1,640	3,000	200	20
West Coast					
Karamea	1,160	330	3,300	105	10
Buller A	4,560	270	2,400	240	15
Buller B	6,350	270	2,600	416	14
Inangahua* A	234	170	2,500	13	13
Inangahua* B	1,000	730	3,000	73	14
Grey	642	550	3,000	48	8
Butchers*	4	270	2,900	0.30	7
Hokitika	352	17,070	9,400	99	7
Haast	1,020	12,730	6,500	193	9
Cleddau	155	13,300	7,000	32	15

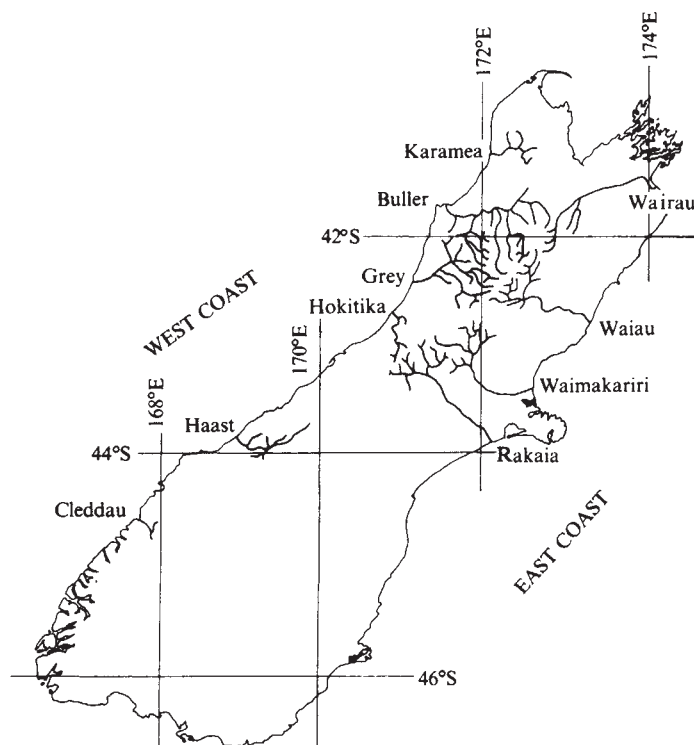
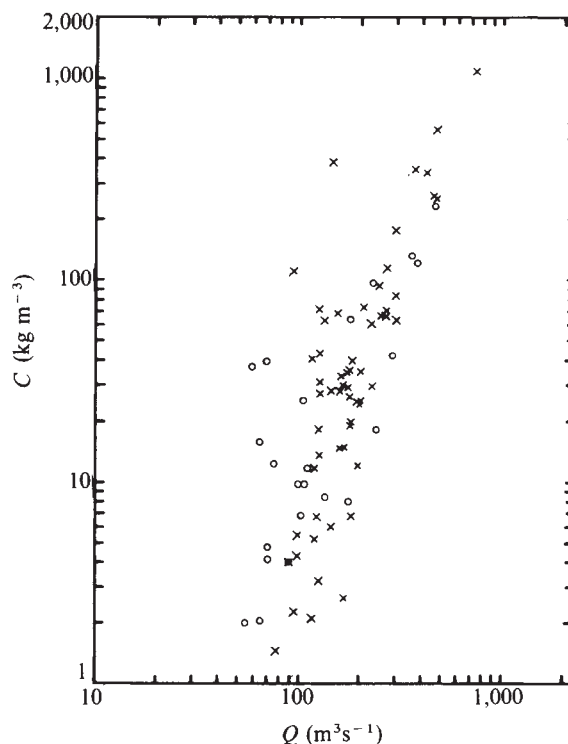
* Minor river.

programme. Unlike water flow gaugings, concentration measurements have mostly been made during floods having peak discharges less than the mean annual flood but ~75% of the sediment is transported by these lower peak discharge, higher frequency events¹.

The algorithm used for the computer calculation of yield was

$$G = (1/1000AT) \sum_{i=1}^n 0.25(C_i + C_{i+1})(Q_i + Q_{i+1}) \Delta t$$

in which A is the catchment area in km², T is the duration of the water flow record in years, n is the number of observations, i is a summation integer and G is the specific suspended sediment

**Fig. 1** Map of South Island, New Zealand, showing the location of major rivers gauged for suspended sediment. Other rivers were either not measured or have sediment storage basins (lakes) upstream of gauging stations.**Fig. 2** Suspended sediment concentration, C plotted against water discharge, Q , for the Rakaia River ($\times$) and the Haast River ($\circ$).

yield in tonnes km⁻² yr⁻¹. At a gauging station the water level is recorded every 900 s and thus Q at the same interval may be derived from a flow rating. It was found to be sufficiently accurate, however, to use a value of 3,600 s for Δt in the above algorithm. It has been suggested² that the absolute errors associated with calculation of sediment yield by methods similar to the above, could be as high as +60% for annual yields. Unfortunately, absolute errors for the data of Table 1 cannot be assessed owing to the absence of measurements made by an alternative precise procedure. The regional consistency of yields demonstrated in Fig. 3 does suggest, however, that the relative error between rivers is small.

Catchments of very different geology, for example, that of the Rakaia River on well indurated sandstone and that of the Haast River on fissile schist, have nearly identical suspended sediment rating relationships (Fig. 2). Furthermore, West Coast rivers (Fig. 1) are steeper, have coarser gravel beds and shorter braided lengths than others in South Island, as they occupy gorges for most of their extent.

Specific yields calculated for East Coast rivers (Table 1) are in general agreement with earlier crude estimates (S. Thompson and J. Adams, personal communication). The world average denudation rate for mountainous terrain is estimated³ to be 1,300 tonnes km⁻² yr⁻¹. Rates for East Coast catchments lie close to this figure. (Bedload and dissolved load is not included in Table 1, but the few measurements available suggest that these components rarely exceed 10% of the total load¹.)

There is a spectacular rise in specific water and sediment yields and in mean annual rainfall south of the Grey River. The specific sediment yield of the Hokitika River is enormous: it is 2.4 times higher than that of the Ching River in China, quoted⁴ as having the largest measured specific yield of major rivers of the world.

A linear multiple regression analysis between sediment yield as the dependent variable and independent variables, including catchment area, mean annual rainfall, mean annual discharge, mean annual flood, 10 yr recurrence interval flood, mean annual runoff, mean annual flood runoff, 10 yr recurrence interval flood runoff, mean basin elevation above sea level, river channel

length, mean catchment slope and percentage forest cover—for West Coast catchments (Table 1) indicated that 95% of the variance in suspended sediment yield for the region is accounted for by mean annual rainfall (Fig. 3). All variables used in the analysis were logarithmically transformed. Geology varies widely from granite to indurated sandstone and mudstone to fissile schist on West Coast and, as implied earlier, variations are apparently of little significance in suspended sediment production.

Current analysis may, however, reveal the relative influence of further parameters.

The simple positive power law relationship between suspended sediment yield and rainfall, P , defined in Fig. 3 by

$$G = 9.02 \times 10^{-11} P^{3.65}$$

confirms previous work which has pointed to this positive trend⁵⁻¹⁰.

In terms of mean annual runoff, R (mm) the relationship is

$$G = 3.51 \times 10^{-8} R^{3.00}$$

which explains 93% of the variance in yield and reflects, in part, the poor water storage capacities of the catchments involved.

The yield/rainfall relationship of Fig. 3 may be typical of other very high rainfall areas such as parts of the Himalayas^{11,12}, New Guinea^{13,14}, Japan¹⁵, Java¹⁶ and Taiwan¹⁷ where large specific yields have been reported. Taiwan is of particular interest, for within the hardwood forest covered, steep Central Range the average specific suspended sediment yield is 13,000 tonnes km⁻² yr⁻¹ which is similar to the average value (14,000 tonnes km⁻² yr⁻¹) for rivers south of the Hokitika River, South Island. Moreover, gross rainfall values¹⁸ exceed 5,000 mm for the higher yielding Taiwan catchments and are comparable to the values of 6,000–7,000 mm for catchments south of the Hokitika River.

The highest specific yield in Taiwan, measured on a minor river¹⁷, is 31,700 tonnes km⁻² yr⁻¹ perhaps the highest known value in the world. But if the relationship in Fig. 3 is valid regionally, then in minor south West Coast drainage basins, where mean annual rainfalls surpass 10,000 mm, yields above 36,000 tonnes km⁻² yr⁻¹ probably exist.

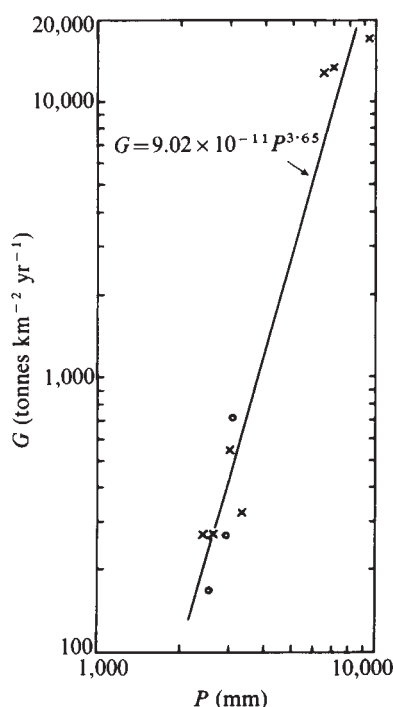


Fig. 3 Mean annual specific suspended sediment yield, G , plotted against catchment mean annual rainfall, P , for major rivers ($\times$) and minor rivers ($\circ$) of the Western Southern Alps, New Zealand.

Predictions of suspended sediment yield as a function of precipitation in semi-arid or arid^{5,9}, subhumid^{7,10}, and tropical^{5,7}, conditions have been given elsewhere. None of these relationships apply to the West Coast region where temperate, maritime conditions exist. In such a climate, for undisturbed catchments, a simple power law expression between yield and rainfall may provide useful estimates of specific suspended sediment yields.

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Response of deep-sea benthonic Foraminifera to development of the psychrosphere near the Eocene/Oligocene boundary

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A dominant feature of present-day thermohaline circulation in oceans is the production of cold Antarctic Bottom Water (AABW) in the Weddell and Ross Seas and along the continental margin off Antarctica. The cold, dense AABW formed at or near the surface sinks to abyssal depths and travels throughout the ocean basins. AABW circulation was suggested to have begun near the Eocene/Oligocene boundary with the development of the psychrosphere (lower cold layer of a two-layer ocean with temperatures $<10^\circ\text{C}$) inferred from ostracode faunal data¹ and oxygen isotopic evidence from closely-spaced samples in DSDP site 277 (ref. 2). Previous studies suggested that the temperature change associated with the development of the psychrosphere caused a crisis for deep-sea ostracodes¹ and deep-sea benthonic Foraminifera³. I consider here the response of benthonic Foraminifera to the development of the psychrosphere.

Benthonic Foraminifera in 15 closely-spaced sediment samples from DSDP site 277 have been examined. Comparison of the faunal data and the oxygen isotopic record reveals that the temperature drop coincides with a pronounced reduction of the frequency of *Epistominella umbonifera*. Few other faunal changes (first or last occurrences) are recorded at the time of the development of the psychrosphere, although 16 faunal changes occurred before and six occurred after the isotopic event. Taxa which exhibit first and last occurrences are all rare forms. I suggest that the psychrosphere may have developed gradually during the middle-late Eocene, culminating in a rapid temperature drop near the Eocene/Oligocene boundary.

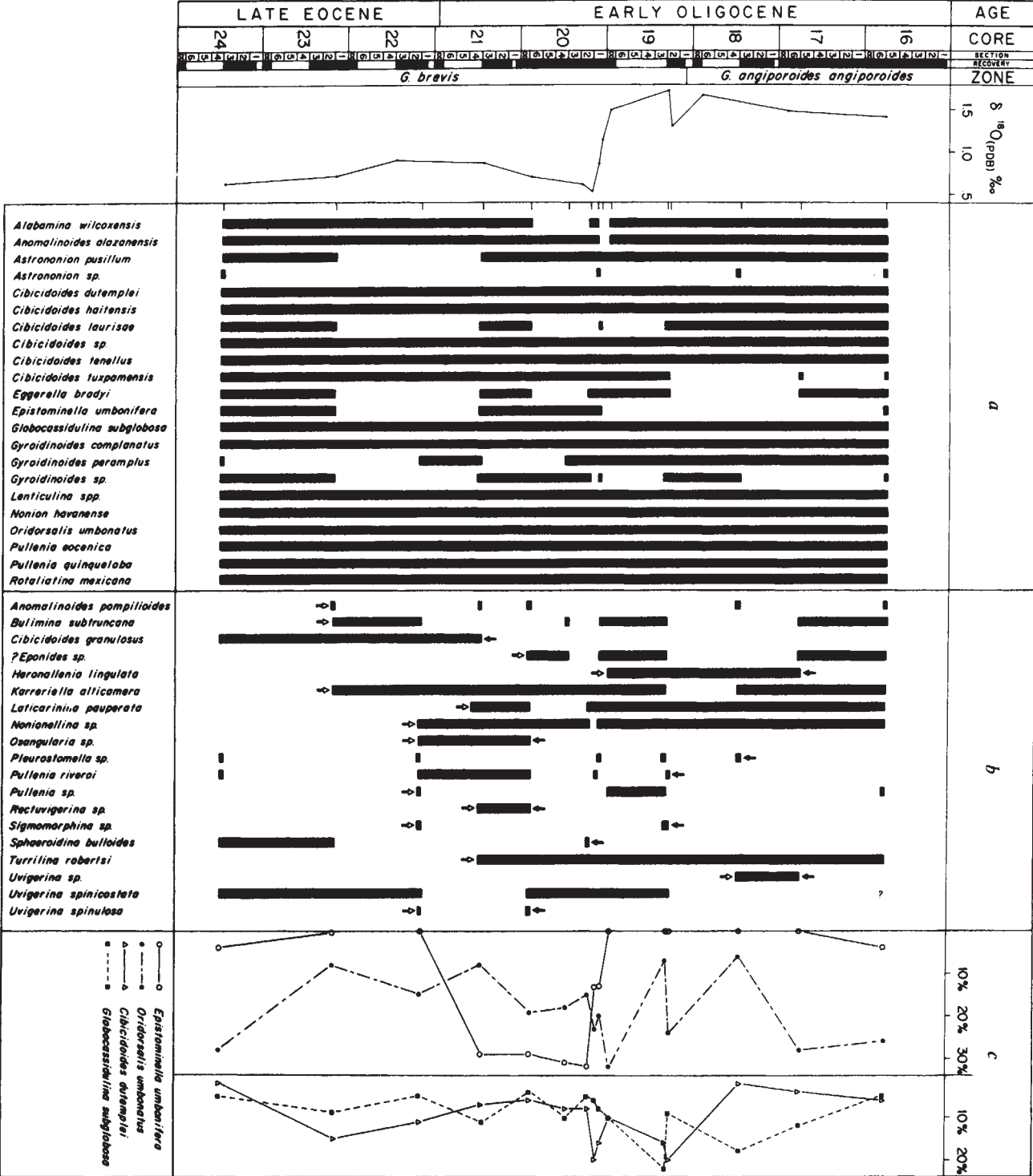


Fig. 1 Summary of benthonic foraminiferal biostratigraphic data from DSDP site 277. The inferred age², core recovery, planktonic foraminiferal zones⁹, and isotopic curve based on mixed benthonic foraminifera² are shown left, with the tick marks indicating the samples examined for the benthonic foraminiferal analysis. Biostratigraphic ranges of 41 taxa are shown: a, taxa that lack first or last occurrences in the sequence; b, taxa that exhibit first or last occurrences. First appearances are marked by open arrows, and last occurrences are marked by solid arrows. c, Quantitative data of four dominant benthonic foraminiferal species.

DSDP site 277 is located on the Campbell Plateau (52°13.43' S, 160°11.48' E, 1,222 m) in sub-Antarctic waters south of New Zealand in the Southern Ocean. The sequence in site 277 consists of continuously cored foraminiferal-rich calcareous sediments⁴. The Campbell Plateau at 38 Myr was at about palaeolatitude 65° S (ref. 5).

The oxygen isotope curve based on mixed benthonic Foraminifera for the late Eocene-early Oligocene (Fig. 1) is assumed to reflect temperature variation of the bottom waters², as the Antarctic continent is considered to have been essentially ice-free during the Eocene and Oligocene⁶. A reduction in bottom-

water temperatures of about 5 °C was recorded in the earliest Oligocene. It has been suggested that the development of the psychrosphere resulted from this temperature change, reflecting a decrease in Antarctic surface water temperature to freezing, the production of the first extensive sea ice and the onset of cold bottom-water production². The initiation of vigorous bottom-water circulation is thought to have caused widespread erosion of sediments, increased calcium carbonate dissolution in some areas, and caused a precipitous drop in the calcium carbonate compensation depth in other areas².

Oxygen isotope analysis of monospecific samples of the benthonic foraminifer *Oridorsalis umbonatus* in this section was recently carried out, and the temperature drop seems to be about 4 °C, (L. Keigwin, personal communication) rather than 4–5 °C as previously thought², but this drop is still significant. The exact stratigraphic position of core 19 samples relative to core 20 is unknown because of incomplete core recovery, and the rate of temperature decrease cannot be precisely determined, although a rapid reduction within 100,000 yr has been suggested².

Species frequencies of four dominant taxa, *C. duteuplei*, *E. umbonifera*, *G. subglobosa* and *O. umbonatus* are shown in Fig. 1c. The inferred temperature drop did not affect the relative abundance of three of these species. In contrast, the inferred temperature change seems to have had a pronounced effect on the relative abundance of *E. umbonifera*. This species is the dominant (29–32%) taxa preceding the isotopic event. A rapid decrease in *E. umbonifera* abundance parallels the isotopic curve during the isotopic change. *E. umbonifera* is not present in cores 19–17, and appears again in core 16 (4%). The water-mass event seems to have affected the relative abundance of this species and changed the composition of the assemblage.

Sixteen faunal events occurred before and six after the isotopic event. Using approximate sedimentation rates of 2.2 cm per 1,000 yr for cores 16–21 and 0.5 cm per 1,000 yr for cores 22–24 (ref. 4), the time interval between cores 16 and 20 is about 1.5 Myr, and the interval between cores 20 and 24 is about 5.5 Myr. The interval from cores 20 to 24 is a compressed section relative to cores 16–19, and may explain the greater number of faunal events before the isotopic event.

Only two faunal changes coincided with the development of the psychrosphere. The faunal changes preceding and subsequent to the isotopic change appear to have occurred gradually. This general faunal pattern is observed in other sequences in the Pacific³ and Atlantic^{7,8}, where faunal changes are found throughout the late Eocene and early Oligocene.

The development of the psychrosphere in the deep sea created a new physicochemical environment, one of the most widespread in the Cenozoic. The occupation of this niche by new taxa would have either resulted from the migration of taxa into the psychrosphere or from the evolution within the psychrosphere of new taxa. The data from site 277 and from the Pacific³ and Atlantic^{7,8} suggest that the faunal turnover close to the inferred temperature change was limited to a few taxa. Following the development of the psychrosphere, the Foraminifera gradually changed during an interval of several million years until a stable assemblage was established. Some forms were unaffected, being present during both the Eocene and Oligocene. The lack of faunal change occurring immediately after the bottom-water temperature drop suggests that the species must have had fairly wide environmental tolerances, and stenotopic species such as those found at present in the deep sea evolved sometime after the development of the psychrosphere. The stenotopic psychrospheric assemblage probably took a long time to develop, perhaps in response to relatively constant environmental conditions in the deep sea. This interpretation is based on the assumption that the isotopic event reflects a temperature decrease as previously suggested^{2,6}, and not a change in the oceanic isotopic composition. No faunal change would be expected if the isotopic event reflected a change in the oceanic isotopic composition.

The isotopic data at site 277 show a pronounced cooling in bottom waters occurring in the mid-early Eocene followed by a steady decrease in bottom temperatures to the Eocene/Oligocene boundary⁶ where the sharp temperature drop is observed. The decrease in bottom temperatures during the middle-late Eocene may reflect the initial and gradual development of the psychrosphere, with the isotopic change near the Eocene/Oligocene boundary representing part of this development and caused by some event that marks a threshold in Southern Ocean palaeocirculation. If the psychrosphere did develop gradually, this would account for the gradual changes in benthonic

Foraminifera observed during the late Eocene before the isotopic drop near the Eocene/Oligocene boundary.

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Late Eocene of Burma yields earliest anthropoid primate, *Pondaungia cotteri*

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In April 1978, a fragment of a primate lower jaw containing the second and third molar teeth was found in late Eocene exposures of the Pondaung Hills about 1 mile north-west of Mogaung village in northwestern Central Burma. This approximately 40-Myr-old specimen is the first fossil primate found in Burma since the fragmentary remains of the controversial earliest anthropoids *Pondaungia cotteri* Pilgrim¹ and *Amphipithecus mogaungensis* Colbert² were recovered more than 50 yr ago. The jaw described here is believed to represent further evidence of *P. cotteri*. Its recovery from undoubted late Eocene exposures coupled with its salient higher primate characters and excellent state of preservation provides the opportunity to substantiate further that the Pondaung primates of Burma are the earliest known record of the Anthropoidea.

The new specimen was recovered with a characteristic late Eocene fauna, including fishes, turtles, sebecid and other crocodylians, brontotheres, amynodont rhinoceroses and anthracotheres, as float on greyish yellow, grey and red claystones. These claystones comprise the uppermost 100 feet of the Pondaung Formation near Mogaung village. Locally, the upper beds of the Pondaung Formation are conformably overlain by a southerly emanating marine incursion called the Yaw Formation, containing a rich foraminiferal and molluscan fauna. This fauna has been correlated with the Ludian (=Bartonian) European marine stage^{3–7} which dates the underlying Pondaung fauna as late Eocene, approximately 40 Myr BP.

The jaw fragment (Fig. 1) is part of a right dentary with M_{2-3} , University of California Museum of Paleontology (UCMP) No. 120377, from UCMP locality V78090, Mogaung Northwest. The animal was about the size of the larger species of living gibbons and probably attained a size and weight (based in part on M_2 length correlation⁸) equal to or greater than that suggested for *Aegyptopithecus zeuxis* Simons or *Adapis magnus* Filhol.

We provisionally assign UCMP 120377 to the Anthropoidea and refer it to *Pondaungia cotteri*. Although the fragmentary nature and chemically eroded enamel surfaces of the holotype of *P. cotteri*¹ (Geological Survey of India, GDI D201-203) make comparisons with UCMP 120377 difficult, we feel the referral is justifiable on both morphological and metrical grounds.

Order Primates Linnaeus, 1758

Suborder Haplorhini Pocock, 1918

Hyporder Anthropoidea Mivart, 1864

?Infraorder Catarrhini E. Geoffroy, 1812

Genus *Pondaungia* Pilgrim, 1927

Pondaungia cotteri Pilgrim, 1927

Revised diagnosis: Gibbon-sized primates with bunodont tetracusate upper molars having weak lingual cingula. The bunodont hexacusate lower molars have only obscure and incomplete ectocingulids. M_{2-3} with many accessory cusps and low ridges effecting a strongly corrugated ('pustulate') occlusal surface.

Range: Two localities in the Pondaung Formation, Late Eocene, Central Burma.

Measurements: As given in Table 1.

Table 1 Measurements (mm) of GDI D201 and UCMP 120377

	GDI D201	UCMP 120377
Depth of jaw below anterior root of M_2 , external (buccal) surface	11.9	16.3
Depth of jaw below anterior root of M_2 , internal (lingual) surface	12.3	17.4
Maximum anteroposterior dimension of crown of M_2	6.1	7.1
Maximum transverse dimension of crown of M_2 (the trigonid is less than 0.1 mm wider than the talonid)	5.9	6.7
Maximum anteroposterior dimension of M_3	7.3	8.3
Maximum transverse dimension of crown of M_3 (= width of trigonid)	5.2	6.0

Immediately recognisable characters of the new specimen are its great jaw depth in relation to molar crown height and its relatively thick mandibular corpus. Nearly all anthropoid primates uniquely share these proportions, which are probably related to increased stresses placed on the mandible during mastication^{9,10}, and their presence in *Pondaungia* is evidence for its inclusion in the higher primates. Simons¹¹ made a similar decision regarding *Amphipithecus*. Figure 2 compares the new specimen with *Amphipithecus* (AMNH 32520) and *Homo*. Furthermore, jaw depth in UCMP 120377 is proportionately greater than in the holotype of *Pondaungia* (see Table 1) which could indicate a juvenile status for the type (P. D. Gingerich, personal communication).

M_2 and M_3 of UCMP 120377 are large, bunodont and multicuspate. M_2 is a rectangle with rounded corners in occlusal outline. Unlike most adapines, notharctines and omomyids, the maximum transverse widths of the trigonid and talonid are about equal. In most living and fossil lower primates the talonid is noticeably wider than the trigonid. The trigonid of UCMP 120377 is only slightly higher than the talonid and there is a prominent, closed, highly corrugated talonid basin. This bunodont tooth has a complicated enamel surface with many

accessory low cusps and ridges with no primary trend. Nevertheless, the six principal (and interpretatively primitive) cusps can be identified and delineated (Fig. 1); all are marginally placed. The strong paraconid lies anterolabial and adjacent to the metaconid, and these two cusps form a twin-peaked crest at the anterolingual corner of the tooth. This arrangement is seen on the M_2 of some omomyids such as *Altanius* Dashzeveg and McKenna¹² and *Tetonoides* Gazin¹³ and on some specimens of the early adapids such as *Pelycodus* Cope. The protoconid and hypoconid are, areally, the largest of the cusps and are evenly rounded (that is, symmetrically bunodont). The entoconid is also evenly rounded but smaller than the labial cusps. A faint cingulum lies anterolabial to the protoconid; otherwise, the lateral faces of the crown of this tooth are smooth.

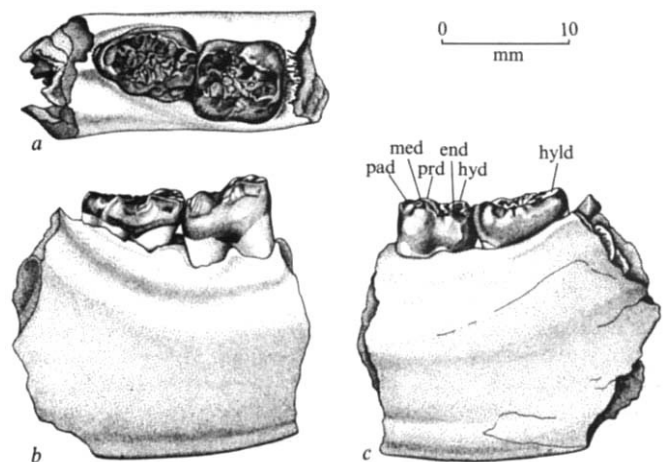


Fig. 1 *Pondaungia cotteri*, right dentary with M_{2-3} , UCMP 120377, from Mogaung Northwest, Central Burma. *a*, Occlusal view; *b*, labial view; *c*, lingual view. Pad = paraconid; end = entoconid; med = metaconid; hyd = hypoconid; prd = protoconid; hyld = hypoconulid.

On the posterior and lateral wall of the M_2 trigonid of *Pondaungia* there is a definable wear facet. This derived (Phase II) wear facet has been called facet 'X' by Kay¹⁴ and facet 11 by Maier¹⁵ and characterises all the living catarrhines and all known fossil catarrhines with the exception of *Oligopithecus*¹⁴. It has not been found in any Palaeogene lower primate and is absent from all living lower primates with the possible exception of some members of the Indriidae¹⁵.

An area interpreted as a worn hypoconulid is a flat, faceted, transversely elongate eminence at the midline of the tooth, forming the posterior border of the central basin. Before wear, it may have been a twin-peaked cusp. Especially in its abraded state, it is much lower than the hypoconid and entoconid. The presence of a hypoconulid on M_2 is a feature which *Pondaungia* shares with the Fayum and later catarrhine primates (other than Cercopithecidae).

The trigonid cusps and basin of the M_3 are slightly higher than the talonid cusps and basin, as on M_2 . Unlike the M_2 , however, the trigonid basin of M_3 is separated from the talonid basin by a continuous high-elevation cristid from metaconid to protoconid. As might be expected from a consideration of the lower molars of other Eocene primates, the paraconid of M_3 is smaller, lower and less distinct from the metaconid than on M_2 . The metaconid is clearly the highest cusp on M_3 . Protoconid and hypoconid are very similar to their homologues on M_2 except that the hypoconid makes a prominent bulge on the labial face of the talonid of M_3 , as it does in many other primates. Also, as on M_2 , a faint and discontinuous cingulum lies labial to the protoconid. No

other cingula are present. The talonid area is elongate posteriorly as in other early primates, and the massive, crescentic hypoconulid—well worn on this specimen—forms an elevated posterior border. The central one of three peaks on the lingual marginal ridge of the talonid basin is identified as the entoconid; it is small compared with the entoconid on *M*₂. The surface of the talonid basin of *M*₃ is even more intricately corrugated than on *M*₂. This dental configuration seems best adapted for crushing and grinding soft plant material and fruits.

UCMP 120377 is thus a mosaic of higher and lower primate characters, with the former more predominant and exemplifying the derived (apomorphic) condition. This new specimen is similar in size and general molar features to the early catarrhine *Aegyptopithecus* Simons¹⁶ from the Oligocene of North Africa, but the conical molar hypoconulids and strong cingulid development in *Aegyptopithecus* coupled with the presence of molar paraconids in *Pondaungia* rule out any close relationship. When *Pondaungia* is compared with Fayum parapihithecoids *Apidium* Osborn and *Parapithecus* Schlosser, the only marked similarities are the bunodonty and lack of cingulid development shared by *Apidium* and *Pondaungia*.

Comparisons with Eocene primates such as omomyoids or adapoids indicate that the lower-molar patterns of *Pondaungia* (and *Amphipithecus*) could be derived from certain species of *Pelycodus* Cope (Early Eocene, North America and Europe). Pilgrim¹ emphasised this in his description of the type of *Pondaungia*. Furthermore, the upper molars of *Pondaungia cotteri* complement this view, for they seem to have pseudo-hypocones in the notharctine manner¹. The more crestiform lower molars of later notharctines in North America and of the adapines in Europe are too specialised (derived) to be considered ancestral, however. Kay¹⁷ also notes that the well developed premetacristid on the *M*₂ of *Pondaungia* which links up with the paraconid to form a complete medial wall for the trigonid is a feature reminiscent of advanced omomyids and early anthropoids, and further suggests that such a trigonid organisation could rule out known European adapids from the ancestry of *Pondaungia*.

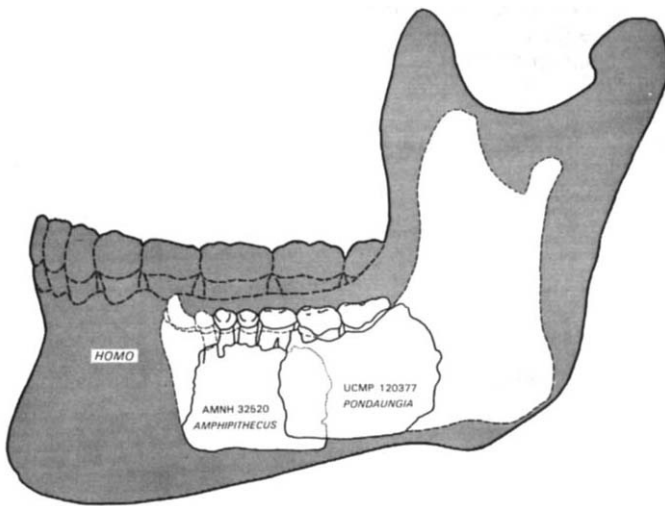


Fig. 2 Size and configuration of the jaw fragments of *Amphipithecus mogaungensis*, AMNH 32520 and *Pondaungia cotteri*, UCMP 120377, compared with *Homo sapiens*. Note how the two Pondaung primates exhibit the same relative jaw proportions as the higher primate *Homo*.

We believe that *Pondaungia* Pilgrim and *Amphipithecus* Colbert are two valid and separate co-existing genera of primates. We also believe that the new specimen of *Pondaungia* was probably a large male in the ancient population that included the type specimen of *P. cotteri* as the smaller females

(see Table 1). This conclusion is supported by the differences in size of adult teeth of males and females documented by Swindler¹⁸ for living primates whose overall size is about the same as *Pondaungia*.

We conclude that *P. cotteri* and *A. mogaungensis* were specialised as deep-jawed primates with bunodont cheek teeth, the former being characterised by corrugated enamel surfaces on its teeth, the latter by smooth enamel. Their anatomy indicates they were at or near the ancestry of the anthropoids, perhaps closest to the Catarrhini. Of course, it is possible that they represent a remarkable convergence towards early anthropoids of an adapoid or omomyoid stock. However, we feel that when the anatomical features of *Pondaungia* (and *Amphipithecus*) are considered in the light of their zoogeographic position and latest Eocene age they seem most likely to represent the earliest known record of the Anthropoidea.

Establishing the precise phylogenetic affinities of *Pondaungia* (and *Amphipithecus*) is difficult, in part because of the scarcity of material. As they are now known, both are too aberrant and specialised to be considered ancestral to any known catarrhine. Their characters, however, do not vitiate Szalay's conclusion^{19,20} that *Amphipithecus* is an advanced early Tertiary lemuriform—adapoid. Similarly, neither *Pondaungia* nor *Amphipithecus* falsify Gingerich's conviction (for example, see ref. 21) that adapoids were ancestral to the anthropoids. From another viewpoint, Delson²² suggests that *Pondaungia* may represent one branch of an omomyid-derived lineage which entered eastern Asia from North America by way of the Bering land bridge; another branch may thence have crossed the Tethys Sea into Africa to give rise to the Fayum (and later) catarrhines. Other hypotheses must be considered, however, pending discovery of more complete fossils from Burma. As the Palaeogene mammalian record of southern Asia is still so poorly known, one might even guess that a primate radicle unrelated to plesiadapiform, adapoid or omomyoid lineages culminated in the late Eocene earliest anthropoids *Pondaungia* and *Amphipithecus*.

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Resistance analysis of sulphur dioxide fluxes to *Vicia faba*

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Before plants respond directly to a gaseous air pollutant, the gas must diffuse into the leaves through stomatal pores or through the cuticle. Some gases are also absorbed or adsorbed on the surface of leaves¹ and this flux elicits no direct plant response. In principle, responses to a pollutant may depend either on the rate at which the gas is absorbed, or on the total quantity absorbed over a period of time. In practice, workers have commonly related responses either to the gas concentration surrounding the plant or to the dose, defined as the product of the gas concentration and the duration of exposure². Plants exposed in an environment where the pollutant flux to leaves is restricted because there is little air movement³ or because stomata are partly closed, are regarded as having the same dose as plants absorbing the pollutant rapidly. Consequently the response to a specified dose depends strongly on the conditions of exposure³. We report here laboratory measurements of fluxes of sulphur dioxide to plants of the field bean, *Vicia faba* L. and relate the observed inhibition of net photosynthesis to this flux. Measured fluxes are analysed to show the several pathways for SO₂ into and on to leaves, and to demonstrate that the internal concentration of SO₂ in the bean leaves was close to zero.

To quantify relationships between concentrations, fluxes and sink strengths, it is convenient to treat the diffusive transfer of gaseous pollutants to plants as analogous to the flow of current in electrical circuits^{4,5}, a method commonly used in the analysis of water vapour and carbon dioxide fluxes^{6,7}. A gaseous flux

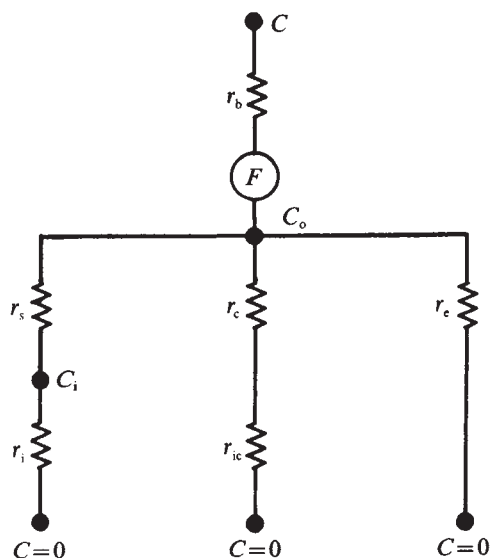


Fig. 1 A resistance analogue of SO₂ uptake by one surface of a leaf. The gas, at concentration C in the exposure chamber, is transferred through a boundary layer resistance r_b to the leaf surface where concentration is C_0 . From the leaf surface there are three parallel pathways to sinks where $C = 0$: (1) through stomata, resistance r_s , into the substomatal cavity where concentration is C_i and subsequently through an internal resistance r_i ; (2) through the cuticle, resistance r_c and through an internal resistance r_{ic} ; (3) by external sorption on the cuticle and on materials on the leaf surface, resistance r_e .

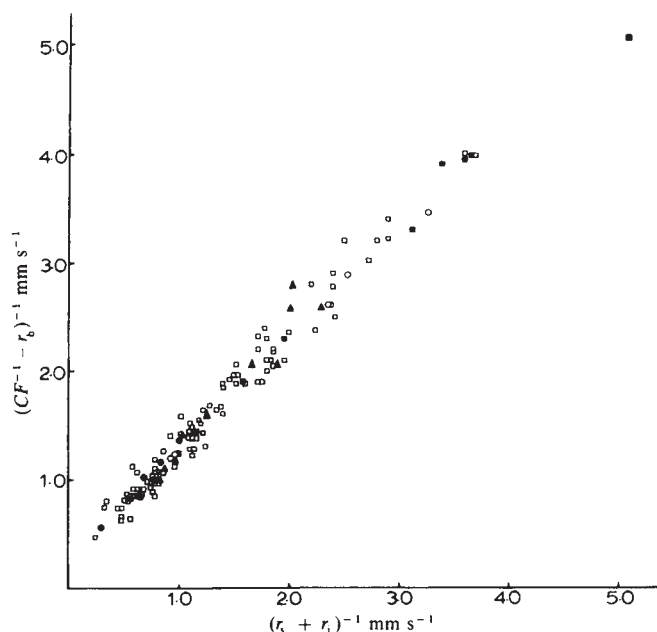


Fig. 2 Variation of $(CF^{-1} - r_b)^{-1}$ with $(r_s + r_i)^{-1}$ for SO₂ uptake by whole plants of *V. faba*. The ordinate is the deposition velocity for SO₂, (flux F /concentration C), modified for boundary layer resistance; the abscissa is the conductance resulting from stomatal (r_s) and internal (r_i) resistances acting in series. SO₂ concentrations: ○, 20 p.p.b.; ●, 40 p.p.b.; □, 75 p.p.b.; ■, 190 p.p.b.; ▲, 300 p.p.b.

(current) is driven by a difference in gas concentration (potential difference) and restricted by diffusive resistances of the leaf boundary layer, stomata and cuticle (resistance). Figure 1 shows a general resistance network for SO₂ uptake by a leaf, indicating parallel pathways through stomata, through cuticle and on to the leaf surface. Inside the sub-stomatal cavity, the SO₂ concentration has the value C_i and further diffusive resistances r_i and r_{ic} describe the restrictions to SO₂ transfer internally as a gas and eventually in solution to a sink where $C = 0$ by definition. In a similar way the resistance r_e limits the rate of surface deposition.

We now describe a method for determining some of the resistances in Fig. 1 from measurements of SO₂ and water vapour fluxes. From Fig. 1, two expressions may be written for the total SO₂ flux density F :

$$F = (C - C_0)/r_b \quad (1)$$

and

$$F = (C_0 - 0)/[(r_s + r_i)^{-1} + (r_c + r_{ic})^{-1} + (r_e)^{-1}]^{-1} \quad (2)$$

using the usual laws for finding the resultant of resistances in parallel. Eliminating the surface concentration C_0 from equations (1) and (2) and rearranging terms leads to

$$(CF^{-1} - r_b)^{-1} = (r_s + r_i)^{-1} + [(r_c + r_{ic})^{-1} + r_e^{-1}] \quad (3)$$

Several terms in equation (3) may be measured or derived. In exposure chambers or cuvettes, the flux density F of gas to unit area of a leaf or plant can be calculated by measuring the concentration difference of gas between inlet and outlet of the chamber and the gas flow rate⁸. The gas concentration C is easily measured. Values of boundary layer and stomatal resistances, r_b and r_s , for SO₂ transfer may be found from analyses of water vapour loss from real and model leaves, making allowances for differences in diffusivity between SO₂ and H₂O (refs 5, 9). The form of equation (3) shows that if measured values of $(CF^{-1} - r_b)^{-1}$ are plotted against $(r_s + r_i)^{-1}$, a straight line graph with slope unity and intercept $[(r_c + r_{ic})^{-1} + r_e^{-1}]$ should result. Because r_i is not known *a priori*, it must be estimated, assuming that it is independent of F and r_s , to obtain the best straight line from a set of measurements.

We measured fluxes of SO_2 to whole plants of *Vicia faba* cultivar Dylan exposed, when they had three pairs of leaflets, in a cubical chamber (side 0.5 m) described elsewhere⁹. The plants were in pots with the soil surface covered to prevent gas exchange. To minimise SO_2 uptake on chamber walls and pots, they were covered with polyvinyl fluoride film and allowances were made in analysing the results for small residual SO_2 fluxes to the film and uncovered plant stems. Simultaneous measurements of concentration differences of SO_2 , H_2O and CO_2 across the chamber allowed fluxes of these gases to be determined and stomatal resistances and rates of net photosynthesis were calculated^{9,10}. The mean boundary layer resistance to SO_2 transfer in our experiments was 100 s m^{-1} .

Each plant was allowed to acclimatise for one photoperiod in the chamber; on the second day, photosynthesis and transpiration in clean air were measured, and on the third day SO_2 was added at fixed concentrations ranging from 20 to 300 p.p.b. ($1 \text{ p.p.b.} = 10^{-3} \text{ p.p.m.} \approx 2.86 \mu\text{g SO}_2 \text{ m}^{-3}$). To change stomatal resistance, irradiance was varied using neutral filters. Values of r_s ranged from 200 to $3,700 \text{ s m}^{-1}$. Throughout the experiments air temperature was $20^\circ\text{--}21^\circ\text{C}$ and relative humidity was 50–60%.

Figure 2 shows the values of $(CF^{-1} - r_b)^{-1}$ plotted against $(r_s + r_i)^{-1}$ when r_i was assumed zero. Regression analysis shows that the points are well fitted ($r = 0.99$) by a straight line with slope 1.02 ± 0.01 and intercept $0.28 \pm 0.02 \text{ mm s}^{-1}$. The reciprocal of the intercept, $3,600 \text{ s m}^{-1}$ corresponds to the resultant of r_e and $(r_e + r_{ic})$ in parallel (Fig. 1 and equation (3)) and it is not possible to calculate the values of these resistances independently from these measurements. However, Spedding¹ found that r_e for surface deposition of SO_2 on filter paper ranged from 2 to $5 \times 10^3 \text{ s m}^{-1}$ and if r_e is similar for bean leaves we conclude that $(r_e + r_{ic})$ must have been of the same order of magnitude as r_e to give a resultant of $3,600 \text{ s m}^{-1}$.

Further analysis of our measurements with different values assumed for r_i reveals that the uncertainty in the estimate of r_i is about 20 s m^{-1} , an order of magnitude smaller than the minimum stomatal resistance we observed. At a typical value of $r_s = 300 \text{ s m}^{-1}$, analysis of the resistance network in Fig. 1 for the resistances derived here suggests that about 90% of the total SO_2 flux to the leaves entered the stomata and 10% was deposited on the surface and/or diffused through the cuticle. Although this latter figure was small in our experiments, it would be necessary to find out how surface and cuticular resistances are influenced by natural 'weathering' and other environmental factors in the field before total deposition of SO_2 on vegetation could be estimated reliably.

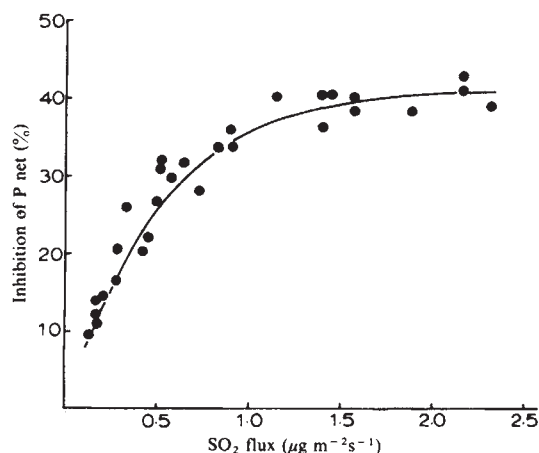


Fig. 3 Variation of the percentage inhibition of net photosynthesis in *V. faba* by SO_2 , relative to clean air, with SO_2 flux entering through stomata. Plants were exposed to SO_2 concentrations ranging from 20 to 200 p.p.b. and the maximum depression in photosynthesis over 2 h was measured.

When plants of *Vicia faba* are exposed to SO_2 , rates of net photosynthesis decrease relative to rates observed in clean air¹⁰. Figure 3 shows the percentage inhibition of net photosynthesis as a function of SO_2 flux density into stomata. In these measurements, plants were exposed for 2 h to SO_2 at concentrations ranging from 20 to 200 p.p.b., and the maximum decrease in net photosynthesis at light saturation was recorded. Fluxes of SO_2 into the stomata were calculated from equations (1) and (2) assuming $r_i = 0$.

By relating response(s) to pollutant flux rather than dose, the influence of different stomatal, surface and boundary layer resistances in different experimental systems is removed. Comparisons of response when fluxes are known may reveal better agreement between responses observed in different laboratories than is apparent from current literature. However, further work is necessary to determine whether specific responses depend on rates of uptake, on uptake integrated over long periods, or on more complex definitions of exposure.

Our measurements and analysis show that at the low and medium concentrations of SO_2 we used, the internal resistance to SO_2 uptake in *Vicia faba* was negligibly small. Consequently fluxes into plants could be estimated from the external SO_2 concentration and the appropriate stomatal resistance. If this result holds generally, the uptake of SO_2 by vegetation in the field or laboratory can be estimated either by relatively simple direct measurements of stomatal resistances and gas concentration or by reference to the extensive literature describing stomatal resistances of many natural and agricultural species. At the biochemical level, our finding that the internal resistance to SO_2 uptake was very small has significant implications for the mechanisms of action of SO_2 within plants.

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'Helpers' in fox society

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Among the Carnivora the presence of non-breeding adults within social groups seems to be widespread, and evidence is accumulating that these individuals sometimes contribute to the survival of young born to other group members who may, or may not, be their relatives^{1,2}. I describe here studies of both wild and captive red foxes, *Vulpes vulpes*, and report that non-breeding vixens occur in fox groups in some habitats. These barren vixens are subordinate to the breeding females, and may feed, guard, groom and play with the cubs of the breeding vixen within their group.

The social organisation of the red fox is still poorly understood, but it is certain that it varies between habitats and sometimes involves monogamous pairs and elsewhere small family groups, and that the proportion of barren vixens also varies between habitats^{3,4}. Around Boars Hill, Oxford, fox groups comprise four or five adults, normally including one male

and several related females, who share a group territory⁵. Of four groups studied in detail in 1973–74, 60% of the vixens were barren, that is, only one or two vixens had cubs in each group. Observations of encounters between barren and reproductive vixens within each group suggested that the breeding vixens were generally dominant amongst the adult vixens of their group. Radio-tracking and direct observation showed that some barren vixens visited breeding earths where they were frequently seen playing with cubs, and circumstantial evidence suggested that they also provided food for the cubs. Where two litters are born within one territory, they may be communally reared in the same earth.

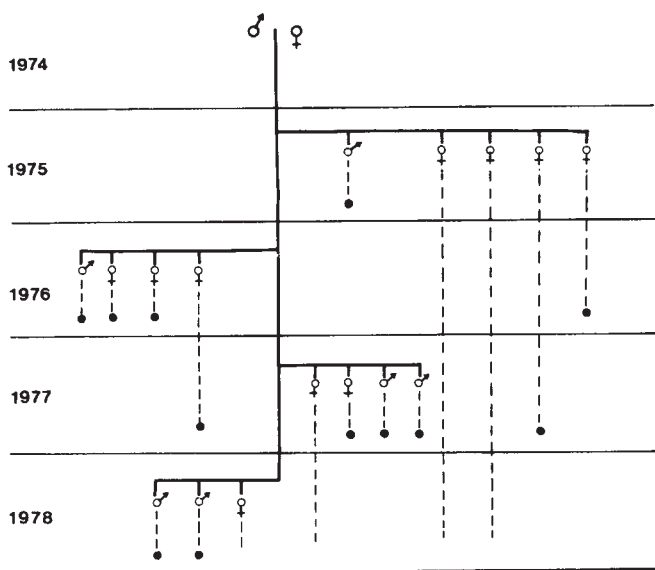


Fig. 1 The genealogy of a captive group of foxes (group I), showing that during 1974–78 only one vixen bred. ● Indicates that individuals were removed from the group. Some non-breeding females acted as helpers.

To study fox reproductive behaviour, two family groups, each mimicking the composition of wild groups, were watched in 1,000-m² enclosures. Group I grew from a pair and their offspring; group II grew from a male and three full sisters of the same age. Hence, both groups comprised related animals. During 1974–79, the groups' composition changed with the addition of cubs each year. All subadult males were removed each autumn, as were some vixens of randomly selected ages.

In eight cumulative years of study of the two groups, the maximum productivity would have been 27 litters. In fact, only seven litters were born (32% productivity, see Fig. 1). Every year except one, only one vixen gave birth to cubs in each group and in every year only one vixen's cubs survived in each group. The male ignored the other females during the mating period and their oestrous condition could not be determined externally. In group I the same vixen bred for 5 yr, throughout which she was consistently dominant to all the others. In group II only one vixen bred in 3 out of 4 yr and two bred in 1 yr, but the identity of the breeding individual changed from year to year. However, in every case the breeding vixen was dominant to the others during the winter in which she bred (see Fig. 2).

In each group some of the non-breeding vixens visited the newborn cubs frequently, but briefly, for the first 3 weeks of their lives; thereafter, they behaved in a way which might promote cub survival by providing food, security and grooming (Table 1). They also attacked wild foxes outside the enclosures while the mother was tending to her cubs, or heavily pregnant. As cubs grew, the litters were split so that some cubs rested with

their mother while others rested with non-breeding females. Table 1 shows that each member of a captive family group often participated in tending to the cubs. However, the percentages given do not necessarily numerically reflect the benefit to the cub that accrued from the behaviour of a particular adult, nor the costs to the adult of its behaviour. This is because the adults did not all 'help' at the same time, nor did they have equal access to the resources they gave to the cubs.

In 1976, the breeding vixen of group 1 suffered a serious injury to a front paw when her cubs were 4 weeks old. On only 2 out of 16 days following the injury was the vixen able to tend to her cubs, but during this period they were found with non-breeding vixens, with whose care they all survived.

In the winter of 1977–78 in group II, the dominance of vixen WP over vixen BE waned, so that by January 1978 they were of equal status (assessed by frequency and direction of amicable and aggressive interactions). Perhaps as a consequence of their equal status, both vixens conceived. By the time the cubs were born, BE was clearly dominant to WP. In the previous year WP had been a successful mother while BE had been a diligent 'helper'. This year BE's cubs were born 13 days before those of WP. During those 13 days BE tended her own cubs. When WP's cubs were born she (WP) repeatedly picked them up and carried them around the enclosure, giving submissive shrieks and adopting submissive posture. Sometimes this 'panic' seemed spontaneous but on other occasions it was prompted by BE's approach. WP's cubs soon succumbed to this treatment. The death of the last cub at 8 days old was followed by a sudden change in WP's behaviour; dropping the corpse outside BE's artificial earth, WP entered and submitted to BE. During the next 10 min WP visited BE's cubs 10 times (having not done so since the birth of her own cubs), and BE visited WP's now empty earth three times. On return from the third trip BE found WP suckling her cubs; the two vixens greeted and BE wandered off leaving WP with her cubs. WP's agitated behaviour had disappeared completely. The two vixens reared BE's cubs cooperatively thereafter.

Table 1 Behaviour of each member of a family group of foxes towards a litter of cubs

	Father Sm	Mother WP	Aunt BE	Aunt WE
Provide food <i>n</i> = 151	41.1	21.2	17.9	19.9
Guard <i>n</i> = 82	2.4	25.6	61.0	11.0
Retrieve <i>n</i> = 30	13.3	43.3	40.0	3.3
Groom/play <i>n</i> = 139	34.5	12.9	32.4	20.1

Values given are the percentage of each of four categories of behaviour undertaken by each member of the group towards the litter (born 19 March) between 5 April, when the cubs began to eat meat, and 18 June 1977. The behaviour categories are providing food, guarding in the earth, retrieving cubs straying from the earth and grooming or playing with cubs.

Each year, in the first 3 weeks following the birth of her cubs, the dominant vixen, irrespective of her identity, furiously attacked the most subordinate of the other vixens, although amicably greeting the more dominant of the subordinates. Each year the more dominant of the subordinate vixens began to feed the cubs earlier than did the more subordinate females. As they began to feed the cubs, each helper suddenly gained 'confidence' during encounters with the male fox. The male frequently played with the cubs and never seemed to threaten their safety. He regularly fed the cubs and, from the moment of their birth, he both fed their mother and cached food around the natal earth.

The female helpers never fed the mother, only the cubs. In spite of their otherwise similar behaviour, foxes apparently differ from jackals¹ in two important respects; jackal helpers feed the breeding female, and male and female jackals are equally likely to act as helpers, whereas most (perhaps all) young male foxes leave the group in their first year^{6,7}.

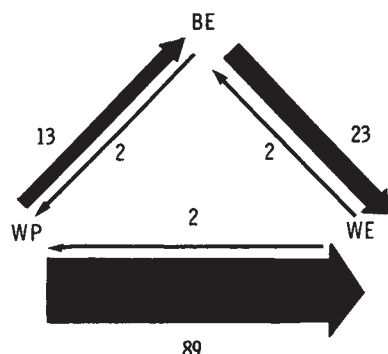


Fig. 2 The number and direction of aggressive interactions between three full sisters within a family group of foxes during the 1977 breeding season (proportion of interactions indicated by arrow thickness). Of 660 interactions recorded between group members, 131 involved aggression between these three females. On this basis a linear hierarchy of WP, BE, WE can be seen.

The mechanism underlying the male foxes' lack of sexual interest in non-breeding vixens is unknown. Two ideas could explain why in the wild the barren vixens remain in the group where they do not breed but do apparently contribute to the survival of another individual's offspring. The helpers may be 'hopeful reproductives'⁸, maintaining a group structure and territory which confer benefits which may at some future time be theirs. The second possibility is that the helpers gain through direct increase in their inclusive fitness (kin selection)⁹ by increasing the chances of survival of cubs to which they are related. These two hypotheses are not mutually exclusive and their relative importance depends, in practice, on the extent to which female membership of fox groups is restricted to relatives. The evidence so far suggests that immigration into established groups is not common and, because helpers could simultaneously be relatives and 'hopeful reproductives', there is no way of deciding whether one or both explanations is correct.

Post-mortem studies of fox reproduction in various habitats have shown differences in the proportion of barren vixens between areas and from one year to the next^{3,10} and between 'age classes'¹¹. These studies suggested that breeding is influenced by density-dependent factors. Reproductive suppression of subordinate vixens in those areas where ecological circumstances permit the formation of multi-female groups could be just such a factor.

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A unique form of locomotion in a stomatopod—backward somersaulting

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Of the diversity of forms of locomotion evolved by life on Earth, one that seems not to have developed to any great extent is rolling or somersaulting. Many cultures claim the existence of 'hoop snakes' that grasp their tails in their mouths and roll downhill, but no known snake can perform this feat¹. Furthermore, although at least one spider (Hamilton, personal communication) when disturbed forms its body and legs into a sphere and rolls down sand dunes, I have been unable to find examples of animals that roll uphill or even over level terrain. Various coelenterates such as *Hydra* and some sea anemones somersault slowly across the substrate by alternating attachment of the pedal disk and tentacles. However, with the exception of human gymnasts and other playful primates, I report here a unique form of locomotion found in the stomatopod *Nannosquilla decemspinosa* (Rathbun, 1910) that consists of a rapid series of backward somersaults.

N. decemspinosa is a small stomatopod growing to a maximum body length of 23 mm. This predatory marine crustacean is found in sand substrates on the Pacific coast of Central and South America. The population studied occurred in the lower third of the intertidal zone on a sand beach on Naos Island, Canal Zone. Individuals excavate 10-15-cm vertical burrows stabilised by a mucus secretion mixed with the sand. When covered by water, the animals sit at the entrance with only their eyes and antennules protruding, waiting for passing prey. When their burrows are exposed at low tide, *N. decemspinosa* close the entrance with a few sand grains and retreat to the bottom of the burrow. *N. decemspinosa* rarely leave their burrows except to dart out and seize prey (usually less than one body length), to locate mates, and when strong wave action destroys their dwelling. When out of the burrow in water, they almost always swim using their five pairs of abdominal pleopods for propulsion. They seldom take more than a few steps on the substrate. One exception is during burrow construction, when they will carry sand 1 or 2 cm from the entrance. Inside the burrow, they use their laterally directed walking legs, as well as their pleopods, in moving quickly up and down. At low tide, if exposed by wave action, they swim if there is as little as a millimetre of water. However, if the sand is well drained and only moist, they are incapable of walking and use a radically different form of locomotion—backward somersaulting. The animal rolls over on its back and brings the telson up over its body, planting it immediately in front of the head, forming a nearly closed loop. Waves of flexion then pass anteriorly from the posterior abdomen and another from the posterior thorax, rolling the animal forward. Finally, the head and carapace are pulled up over the rest of the body and flip over the top so that the animal is once again lying stretched out on its back. At this point the telson is again flexed anteriorly and a new cycle begins (Fig. 1).

The animal continues a series of backward somersaults, generally moving in a straight line until it encounters an obstacle or water sufficiently deep for swimming, or until it becomes fatigued. Although the longest track recorded was just over 2 m, most animals travelled somewhat less than 1 m. The distance covered by each somersault is basically the length of the body. As the average adult is 20-23 mm long, the animals made 20-40 consecutive somersaults.

Body length and speed were positively correlated ($r = 0.857$, $n = 30$, $P < 0.001$) (Fig. 2). Thirty animals ranging in length from 12 to 23 mm were brought into the laboratory. Each was

placed on a level, damp sand substrate and the total length and time taken for a somersaulting track recorded. The sand surface seemed identical to that of the beach at low tide. Animals were started by dropping them onto the sand from a height of 5 cm. All immediately began somersaulting. Curved tracks and those shorter than 15 cm were not scored and the animals were retested several minutes later. In this series of tests, speeds ranged from 1.5 to 4.5 cm s⁻¹. In other trials, the maximum speeds recorded were 5.6 cm s⁻¹ for a 20-mm female and a 23-mm male.

To determine whether the animals take any particular orientation with respect to the beach when somersaulting, several individuals were dug out of their burrows at low tide (during both the day and the night) and were dropped immediately onto smooth sand near their burrow. The initial direction taken was then noted. Direction taken was not significantly different from random orientation, although there was a tendency for animals starting uphill to curve more often than those heading downhill. However, *N. decemspinosa* could somersault up slopes as steep as 10°, and in the laboratory on a wooden substrate some animals could climb even a 30° incline.

This unique form of locomotion in *N. decemspinosa* seems to have evolved in conjunction with other morphological adaptations related to inhabiting fine sandy substrates. The *Nannosquilla* body type is among the most slender, dorsoventrally flattened and loosely articulated of any stomatopod². This allows it to use small-diameter burrows requiring a minimum of time, effort and stabilising mucus for construction. It is parti-

cularly important to *N. decemspinosa*, as this species frequents sandy beaches where burrows are commonly destroyed by wave action. *N. decemspinosa* also is capable of making jack-knife turns in a tube barely larger in diameter than its own width. When digging, it proceeds to the bottom of its burrow headfirst, grabs a load of sand in its maxillipeds, makes a U-turn and carries the sand to the surface. Thicker and more tightly articulated stomatopods, such as the cavity-living gonodactylids or squillids inhabiting U-shaped burrows, cannot turn 180° in such narrow quarters and would require a relatively larger diameter burrow.

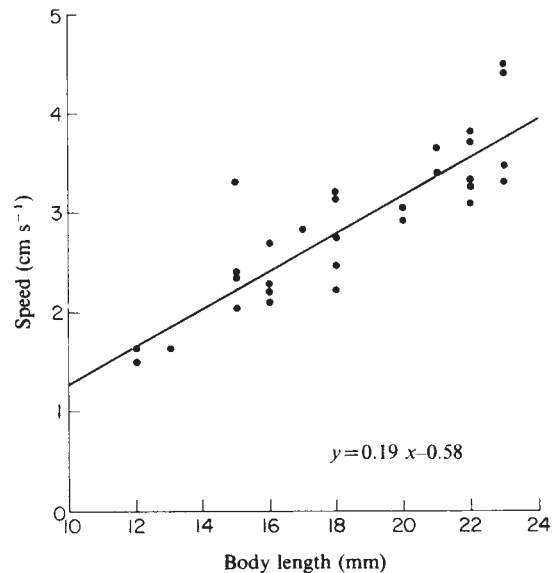


Fig. 2 Speed of backward somersaulting as a function of body length in *N. decemspinosa*. Each point represents a single individual somersaulting on smooth sand. Body length and speed were positively correlated ($r = 0.857$, $n = 30$, $P < 0.001$).

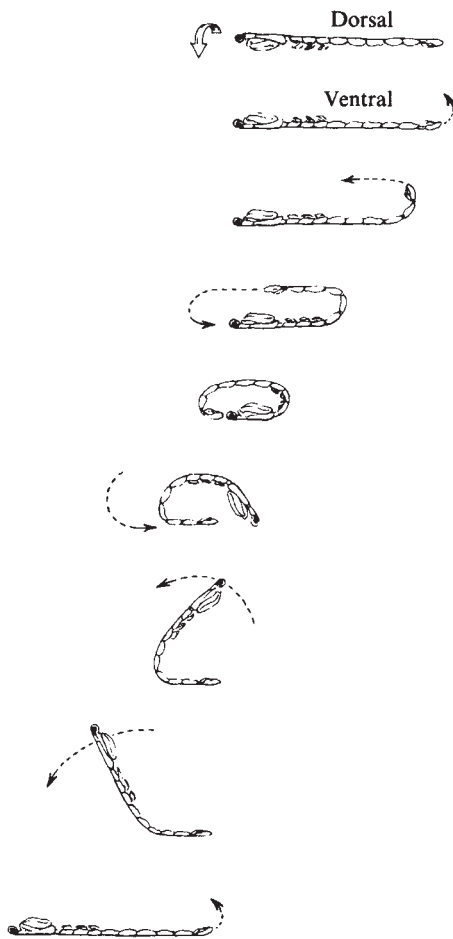


Fig. 1 One cycle in the backward somersaulting of the stomatopod, *N. decemspinosa*. If the animal is dorsal side up, it rolls over onto its back. It then draws the telson up over its head, rolls forward until the head and thorax are flipped up over the body, and lands with the body again stretched out on its back. Animals may complete 20–40 consecutive somersaults.

Most intertidal stomatopods, when exposed on dry land, are capable of crawling using their walking legs. A few can also inch their way forward using their spined telsons to push themselves along. The long, slender body of *N. decemspinosa* offers too much resistance in contact with the substratum for its legs to pull it forward, and the laterally directed legs are too short and weak to lift the body off the sand to reduce friction. The alternative method is somersaulting. Although the turning movements used in the burrow are different from those used while somersaulting (turns in the burrow are made headfirst while somersaults are telson-first), the flexibility essential for making U-turns in the burrow is also required for somersaulting. Also, the dorsoventral flattening provides a broad, flat dorsal surface that gives the animal considerable stability while rolling. Most stomatopods have more rounded dorsal surfaces in cross-section. Thus, although the evolution of the long, slender body makes it impossible for *N. decemspinosa* to move on dry surfaces by walking or crawling, it does provide the flexibility needed for somersaulting.

The data suggest that there is no initial orientation with respect to the slope of the beach during somersaulting. However, the animals are proceeding in an anterior direction and can see objects in their path. Several times, somersaulting animals stopped before running into objects, but there was no indication that they could alter their course to avoid obstacles. When animals were rolling along parallel to the beach, they frequently curved downhill towards the water. This was presumably due to gravity pulling them in that direction, much as a hoop rolled across a slope will curve downhill. Thus, although animals somersault in any direction, even uphill, and do not seem to orientate in any particular direction, there is a tendency to move down the beach towards water. As they are

most likely to be exposed by wave action when the tide is coming in or going out, movement of only 1 or 2 m down the beach would probably bring them back into contact with water.

I have observed one other species of *Nannosquilla* (not described from the Atlantic coast of Panama), and it also somersaulted when exposed. Similar morphology in other *Nannosquilla* species would suggest that they may also possess this ability. Interestingly, another species of stomatopod, *Acanthosquilla digueti*, that is found in micro-sympatry with *N. decemspinosa* and has a very similar body shape, does not somersault. When exposed, it turns over on its back as does *Nannosquilla* and flips its telson up over its head, but it does not continue the motion completing the somersault, and simply flops back and forth. I have never seen it make two consecutive somersaults. Whether other lysiosquillids with similar morphologies can locomote by somersaulting is unknown.

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Protective immunisation of mice using cell surface glycoprotein from *Trypanosoma cruzi*

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The parasitic protozoan *Trypanosoma cruzi* is the causative agent of the chronic debilitating Chagas' disease in man. It is estimated that at least 12 million people in South and Central America are infected with *T. cruzi*, for which there is so far no suitable chemotherapy^{1,2}. The development of vaccines using killed or attenuated whole organisms is hindered by the presence of endotoxin-like material in the protozoa³, and the possible existence of antigens in *T. cruzi* which cross-react with, and thereby stimulate immunity against, mammalian heart and nervous tissue^{4,5}. Because autoimmunity induced in this way has been thought to cause the cardiac and neural lesions characteristic of the chronic stage of the disease^{6,7}, it has been assumed that immunisation based on a whole organism vaccine might carry the risk of itself inducing symptoms of the disease. Recently an integral membrane glycoprotein of molecular weight 90,000, was isolated from the cell surface of *T. cruzi*. It is the only major cell surface glycoprotein, it is present throughout the life cycle of the parasite⁸, and it does not undergo antigenic variation⁹. We have successfully protected mice against acute lethal *T. cruzi* infection by vaccinating them with glycoprotein in combination with adjuvants. Moreover this glycoprotein does not seem to be the one carrying the antigens causing autoimmune antibody formation.

The prophylactic immunisation of mice with *T. cruzi* glycoprotein in conjunction with either Freund's complete adjuvant or saponin as adjuvant against a subsequent lethal challenge with Y strain *T. cruzi* is illustrated in Fig. 1. (The successful use of saponin as an adjuvant for immunising mice with various whole *T. cruzi* vaccines has been described previously¹⁰.) Unimmunised C57BL/6 mice (Fig. 1a) all developed high blood parasitaemias and died (mean survival time, MST, 24.2 ± 0.75 days). Immunisation with Freund's complete adjuvant alone was not protective as judged by either blood parasitaemia or survival time (MST 25.2 ± 0.5 days). After immunisation with glycoprotein alone all mice died but parasitaemia was delayed and

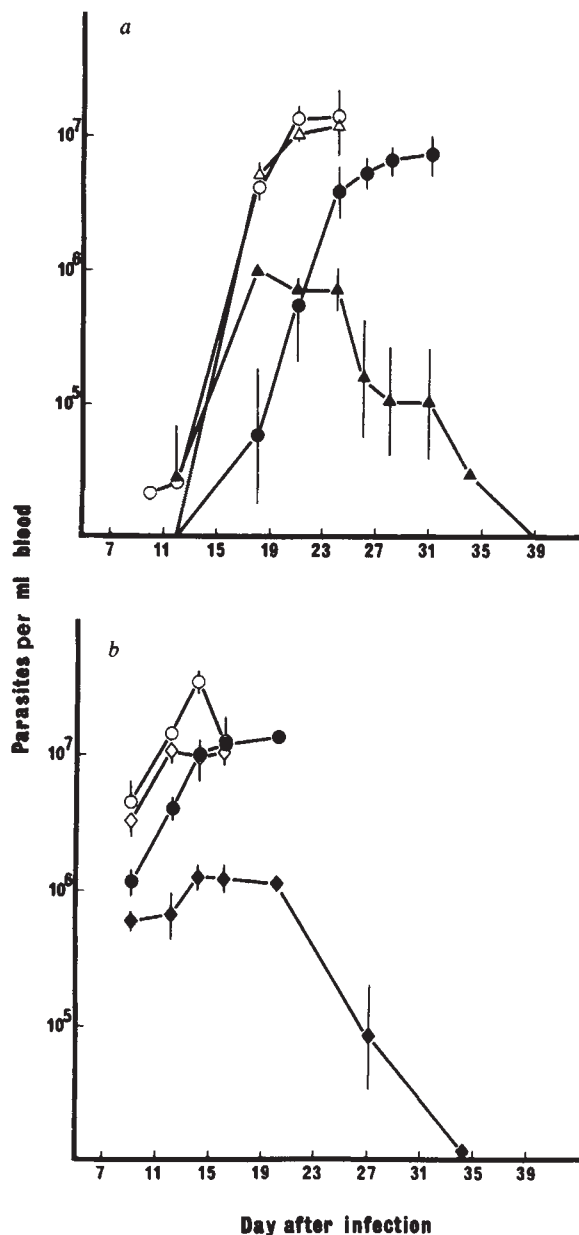


Fig. 1 Protection of mice against acute lethal *T. cruzi* infection by immunisation with *T. cruzi* glycoprotein and adjuvant. a, Female C57BL/6 mice were immunised s.c. with 100 µg glycoprotein emulsified in an equal volume of Freund's complete adjuvant (Difco) (▲), glycoprotein alone (●) Freund's complete adjuvant alone (△), or were untreated (○). Immunisation was repeated after 14 days and, 14 days after the second injection, all mice were challenged s.c. with 5×10^3 Y strain blood trypomastigotes from mouse passage. At the times shown, 10-µl blood samples were taken, mixed with 0.87% ammonium chloride, and parasites counted using a phase contrast microscope. There were five mice in each group and the vertical lines shown represent $\pm$ s.e.m. b, Female (CBA x C57BL/6)F₁ mice were immunised as in (a) except that 50 µg of Quilleya saponin (SPL Boake Roberts and Co.) was substituted for Freund's complete adjuvant. Mice were challenged s.c. with 7.5×10^4 Y strain trypomastigotes. ◆, 100 µg glycoprotein plus 50 µg saponin; ●, glycoprotein alone; ◇, saponin alone; ○, untreated control. There were five mice in each group and the vertical lines represent $\pm$ s.e.m. Glycoprotein: for the above experiments a clone of epimastigotes (Wel Tryp Y₂-C₁) was used as a source of glycoprotein. Culture conditions and glycoprotein preparation using the lectin from *Lens culinaris* for affinity chromatography were described previously⁸ except that the non-ionic detergent Renex 30 (Honeywell Atlas) was used to solubilise the glycoprotein and throughout the fractionation procedure. Glycoprotein was precipitated by adding 3 vol. of ethanol and incubated at -20°C for 48 h. The precipitated glycoprotein was resuspended in phosphate-buffered saline for immunisation.

Table 1 Absorption of autoreactive human Chagas' serum with whole *T. cruzi* or glycoprotein fraction

Serum	IFA titre against	
	Heart*	<i>T. cruzi</i> †
Chagas' serum unabsorbed	1:512	1:4,096
Chagas' serum absorbed <i>T. cruzi</i> ‡	1:64	1:8
Chagas' serum absorbed glycoproteins§	1:512	1:1,024
Normal human serum	1:8	1:16

* IFA (indirect fluorescence assay) for anti-heart reactivity as for Fig. 2.

† Air-dried smears of *T. cruzi* epimastigotes from *in vitro* culture were substituted for heart in IFA.

‡ Absorbed with 45 mg lyophilised epimastigotes ($\approx 4.5 \times 10^9$ organisms per ml serum; 1 h at 37 °C and then overnight at 4 °C.

§ Absorbed with glycoprotein (500 µg protein, $\approx 10^{10}$ organisms per ml serum; 1 h at 37 °C and then overnight at 4 °C.

survival prolonged (MST 31.2 ± 1.2 days). The combination of glycoprotein with Freund's complete adjuvant significantly reduced parasitaemia levels and allowed all mice to eliminate parasites from the blood, as judged by microscope count, and survive. The same pattern was seen in (CBA $\times$ C57BL/6) F₁ mice where saponin was substituted for Freund's complete adjuvant (Fig. 1b): mice immunised with glycoprotein in conjunction with saponin all cleared their blood parasites and survived whereas those receiving either glycoprotein or saponin alone died with high blood parasitaemias. In the experiment shown the degree of protection afforded by immunisation with glycoprotein alone was less than seen in the previous experiment (Fig. 1a), however, in other experiments it has been more marked. It is clearly variable and, being relatively weak, its protective capacity probably depends on the virulence of the individual experimental challenges.

A characteristic manifestation of the autoimmunity associated with Chagas' disease is the development of autoantibody directed against endocardium, vascular and interstitial tissue in mammalian heart (EVI antibody⁴). To determine whether the glycoprotein antigens shared identity with the tissue antigens against which *T. cruzi*-induced autoimmunity is directed, serum from a human Chagas' disease patient showing characteristic EVI activity (Fig. 2) was absorbed with glycoprotein. Table 1 shows that whereas absorption of serum with lyophilised whole *T. cruzi* epimastigotes significantly reduced anti-EVI activity and completely abolished anti-*T. cruzi* activity, absorption with glycoprotein did not affect the anti-EVI titre and was less

effective at reducing anti-*T. cruzi* activity. These data indicate that the glycoprotein and the relevant tissue antigens do not cross-react, and also confirm that the glycoprotein represents only a part of the antigenic profile of the whole organism. Five further sera from human Chagas' disease patients (mean EVI titre $\log_2 6.7 \pm 0.4$) have been similarly absorbed and again absorption with glycoprotein did not significantly reduce EVI activity (titre $\log_2 6.2 \pm 0.5$), whereas absorption with whole *T. cruzi* epimastigotes did (titre $\log_2 4.3 \pm 0.8$).

These results together with the protection data suggest that the glycoprotein could be a candidate antigen for a vaccine against American trypanosomiasis. Immunisation with glycoprotein alone was partially protective as evidenced by slight, but significantly, lower blood parasitaemias and prolonged survival times. Further research should establish whether the adjuvant requirement is absolute for optimal immunisation with the glycoprotein and, if so, whether clinically acceptable adjuvants may be substituted. We also require to know whether any glycoprotein-based vaccine is capable of achieving sterile immunity, and being prophylactically effective against later development of chronic forms of Chagas' disease. It has been proposed that the late pathological manifestation of chronic Chagas' disease develops as a result of tissue damage inflicted during the acute phase of infection¹¹. Any reduction in the acute phase may, therefore, in addition to reducing the mortality associated with acute infection, also decrease the incidence or severity of the chronic form of the disease.

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Interferon enhances 2-5A synthetase in embryonal carcinoma cells

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Mouse teratocarcinomas provide a useful model of mammalian differentiation, because the malignant embryonal carcinoma (EC) stem cells of such tumours may produce various differential cell types *in vivo* or *in vitro*¹. Many EC cell lines have now been established and classified on the basis of their ability to differentiate *in vivo* into cell types characteristically derived from any of the three germ layers^{2,3}. There is convincing evidence that EC cells can neither produce interferon, nor respond to it by becoming resistant to virus, whereas differentiated cells derived from EC lines behave normally in both respects⁴. We investigated the lack of responsiveness of EC cells towards interferon by measuring the levels of two double-stranded RNA-dependent enzyme activities recently shown to be enhanced by interferon^{5,6}.

We report here that on treatment with interferon, EC cells show increased 2-5A synthetase levels comparable to those found in differentiated cells, while there is little or no effect on kinase activity in EC cells, in contrast to their differentiated counterparts.

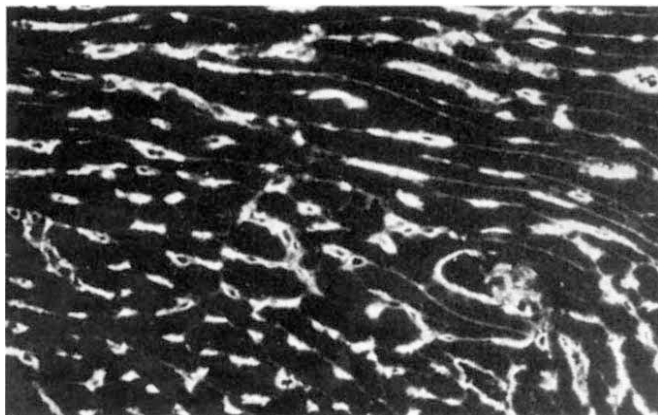


Fig. 2 Characteristic EVI reactivity of human Chagas' serum against mammalian heart tissue. Cryostat sections of mouse heart (5 µm thick) were prepared and stored at -20 °C. They were incubated with serial dilutions of test serum for 20 min at room temperature, washed, developed with a 1 in 20 dilution of FITC-labelled rabbit anti-human IgG (Miles Yeda) for 20 min at room temperature and, after a final wash, examined using a fluorescence microscope. Normal human serum in identical conditions gave no detectable staining.

The conditions of culture and sources of the cell lines used have been described elsewhere^{7,8}. Briefly, all the EC cell lines used are derived from the transplantable ascitic form of the OTT 50-60 teratocarcinoma isolated by Stevens⁹. PCC3/A/1 and PCC4AzaR1 are multipotent lines which give rise to a variety of differential cell types *in vivo*, and in appropriate conditions *in vitro*⁸. The line 3/A/1-D-1 M is a cloned differentiated derivative of PCC3/A/1. These non-malignant cells characteristically give rise to bone *in vivo*. As well as this line, mouse L929 cells were used as differentiated controls which respond to interferon by the establishment of antiviral state.

The first 2-5A synthetase, forms oligonucleotides of the series pppA(2'p5'A)n—collectively referred to as 2-5A—which potently inhibit protein synthesis *in vitro* and *in vivo*, probably by activation of a nuclease¹⁰⁻¹⁴. The kinase studied, phosphorylates a protein of molecular weight 67,000, a 38,000-MW protein which is almost certainly the small subunit of initiation factor eIF2, and exogenous histones^{5,15-18}.

The 2-5A synthetase and the protein kinase were assayed after partial purification on columns of poly(I).poly(C)-Sephacrose as elsewhere described^{5,6}. The column-bound synthetase is stable, thus providing a convenient method for the synthesis of 2-5A free of the enzyme responsible for its degradation⁵. Similarly, the poly(I).poly(C)-Sephacrose-bound protein kinase is stable and free of any phosphatase activity which may be present in the cell extracts⁵.

Figure 1 shows a typical comparison of the kinase activity in PCC3/A/1 cells and their differentiated derivatives 3/A/1-D-1 M, together with L929 cells with and without interferon treatment. The enhanced phosphorylation of the 67,000-MW polypeptide is apparent in cell fractions from L929 and 3/A/1-D-1 M cells (Fig. 1, lanes 2, 6) but not from PCC3/A/1 cells (Fig. 1, lane 4). As a test for enhanced kinase activity, phosphorylation of calf thymus histones was analysed by the poly(I).poly(C)-Sephacrose-bound enzyme fractions. As expected of interferon-treated cell extracts, there was an enhanced phosphorylation of one of the major histone bands in fractions from L929 and 3/A/1-D-1 M cells (Fig. 1, lanes 8, 12) but not from PCC3/A/1 cells (Fig. 1, lane 10). Phosphorylation of the 67,000-MW protein and added histone by the poly(I).poly(C)-Sephacrose-bound enzyme(s) fractions (Fig. 1) from extracts of cells untreated with interferon was also apparent although to a lesser extent, as previously observed^{5,6}. To quantitate the increase in kinase activity on interferon treatment, histone bands were cut out of the dried gel (using the autoradiograms as a guide) and counted in a liquid scintillation counter. There was no apparent increase in the phosphorylation of histones by enzyme fractions from two EC cell lines (PCC3/A/1 and PCC4 AzaR1) after interferon treatment, whereas L929 and 3/A/1-D-1 M extracts showed, respectively, 2.6- and 2.8-fold increases (Table 1).

In contrast to these differences in kinase induction, both undifferentiated and differentiated cells showed similarly enhanced levels of 2-5A synthetase after interferon treatment (Table 1). The level of 2-5A synthetase in different extracts was quantitated by the capacity of the poly(I).poly(C)-Sephacrose-bound enzyme fraction to synthesise 2-5A on incubation with ATP. The heat-stable (95 °C for 10 min) inhibitor of cell-free protein synthesis generated on incubation of enzyme fractions from teratocarcinoma cells showed the same spectrum of resistance and sensitivity to alkali, micrococcal nuclease, snake venom phosphodiesterase and bacterial alkaline phosphatase as the corresponding material synthesised with extracts from L929 cells or rabbit reticulocytes (data not shown, similar to those in ref. 21), thus confirming that the inhibitor synthesised was the 2'5'-linked oligoadenylic acid triphosphate.

Treatment of cells with interferon induces both antiviral and anticellular effects^{24,25}. These were assayed by the resistance to vesicular stomatitis virus (VSV) infection, and by inhibition of cell multiplication. As interferon does not induce an antiviral state in EC cells⁴, it was of interest to attempt to correlate the induction of 2-5A synthetase in these cells with the anticellular

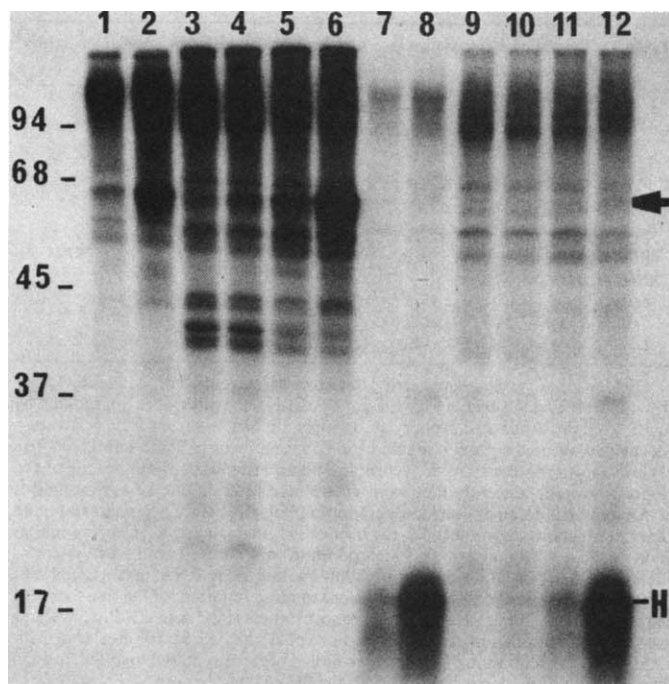


Fig. 1 Protein kinase activity of partially purified enzyme(s) fractions from control and interferon-treated cells: electrophoretic analysis on polyacrylamide (10%) slab gels containing SDS^{19,20}. An autoradiograph of a stained, dried gel is shown. Chromatography on columns of poly(I).poly(C)-Sephacrose, preparation of cell extracts and assay of the 67,000-MW polypeptide and histone kinase activities were as described previously^{5,10,21}. PCC3/A/1, PCC4 AzaR1 and PCC3/A/1-D-1 M cells were grown in Dulbecco modified Eagle's medium containing 15% fetal calf serum (Flow) in a 10% CO₂ humidified atmosphere at 37 °C. L929 cells were grown in basic medium (Eagle) with Earle's salts and 10% calf serum in otherwise similar conditions. Cell extracts (5,000g supernatant) were from control (lanes 1, 3, 5, 7, 9, 11) and interferon-treated (250 units ml⁻¹, 17 h) (lanes 2, 4, 6, 8, 10, 12) mouse cells. Cell lines: L929, 1, 2, 7, 8; PCC3/A/1, lanes 3, 4, 9, 10; PCC3/A/1-D-1M, lanes 5, 6, 11, 12; 67,000-MW polypeptide kinase, lanes 1-6. Poly(I).poly(C)-Sephacrose-bound enzyme(s) fractions from control and interferon-treated cells were incubated for 60 min at 30 °C with 0.15 mM [γ -³²P]ATP (7.1 Ci mmol⁻¹, Radiochemical Centre) in buffer containing glycerol (10 mM HEPES, 90 mM KCl, 10 mM magnesium acetate, 7 mM 2-mercaptoethanol and 20% glycerol, v/v). Phosphorylation of histone, lanes 7-12: poly(I).poly(C)-Sephacrose-bound enzyme fractions from control and interferon-treated cells were incubated (60 min, 30 °C) with 1 mM ATP. Calf thymus histone (Type IIA, Sigma, 1 mg ml⁻¹) and [γ -³²P]ATP (0.15 mM as above) were added and incubation continued for 60 min. All the samples (1-12) were heated (95 °C, 10 min) in electrophoresis sample buffer containing SDS and supernatants equivalent to 0.3 mg of cell extract applied to the gel. The position of the 67,000 MW polypeptide (arrow) and histone (H) are indicated to the right of the gel. The numbers to the left give the MWs of protein markers in thousands: phosphorylase B, 94; bovine plasma albumin, 68; ovalbumin, 45; chymotrypsinogen, 37; myoglobin, 17.5. The interferon used was an electrophoretically homogeneous preparation of specific activity 2×10^9 units per mg, and was prepared and provided by E. De Mayer.

effects of interferon. As expected, no resistance to VSV was induced in EC cells by interferon, whereas L929 and 3/A/1-D-1 M cells showed complete resistance to viral infection (Table 2). Similarly the interferon-mediated inhibition of cell growth in L929 and 3/A/1-D-1 M cells (58% and 42%) was not observed in EC cells, even at high concentrations of interferon (Table 2). Interferon therefore induces the 2-5A synthetase in EC cells without consequent antiviral or anticellular effects. Previous observations on the response of embryonic tissue to interferon have demonstrated a lack of cell growth inhibitory activity²⁶ and a low antiviral response which, however, increases with age²⁷.

Table 1 Levels of 2-5A synthetase and protein kinase(s) in interferon-treated and control cells

Cell type	2-5A synthetase (pmol mg ⁻¹ h ⁻¹)		Histone kinase (cpm mg ⁻¹ h ⁻¹)	
	Control	Interferon-treated	Control	Interferon-treated
EC cells				
PCC3/A/1	<2	225	664	697
PCC4AzaR1	<2	575	987	953
Differentiated cells				
PCC3/A/1-D-1 M	2	418	1,346	3,774
L929	8	562	1,280	3,360

Histone kinase assays were carried out on extracts from control and interferon-treated cells as described in Fig. 1 legend. Relative activities were quantitated by cutting out the bands of phosphorylated exogenous histones from dried gels using a developed autoradiogram as a guide and counting them in liquid scintillant. The figures shown represent c.p.m. incorporated into histones per hour per mg of cell extract. Protein concentrations were determined by the Folin Lowry method²². 2-5A synthetase activity was measured using poly(I). poly(C)-Sepharose-bound enzyme fractions to synthesise 2-5A from ATP⁶ and assayed by means of inhibition of ¹⁴C-labelled amino acid incorporation in a mouse L cell-free system as described^{5,6}. Translation of turnip yellow mosaic virus RNA in an L cell-free system was carried out in similar conditions to those reported for the translation of encephalomyocarditis RNA^{20,23}. The concentration of 2-5A in AMP equivalents was calculated from absorbance on the basis of A_{260} of $15.6 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ for AMP. The concentration of a standard solution of 2-5A required to inhibit protein synthesis by 50% corresponded to about 0.5 nM as previously reported^{6,10,21}. 2-5A synthetase activities were calculated on this basis and are given as units corresponding to 1 pmol of 2-5A synthesised per hour per mg of cell extract.

Our present results agree with these observations and clearly show that there is no correlation between the induction of 2-5A synthetase and the establishment of an antiviral state, or anticellular effects in undifferentiated EC cells. However, one cannot exclude the possibility that the induction of the 2-5A synthetase, although not conferring an antiviral state in EC cells, might nevertheless have such a role in normal tissue, in conjunction with other factors such as enhanced kinase activity. Moreover, although the 2-5A synthetase activities in EC cells and differentiated cells may be the same, the absence of the 2-5A activated nuclease would presumably counter the *in vivo* effects of enhanced synthetase activity. We therefore measured the rate of degradation of VSV mRNA into acid-insoluble material by cell extracts incubated in the presence and absence of 2-5A (data not shown, similar to Fig. 6 in ref. 28). Enhanced nuclease activity was present in all cell extracts when 2-5A was added, suggesting that the 2-5A-dependent nuclease is present in both EC and differentiated cells. Nevertheless, it is still possible that the synthesis of 2-5A or its action could be inhibited in EC cells. In addition, the levels of synthetase activity measured here probably do not accurately reflect the *in vivo*

activity of the enzyme, but rather its physical presence in, perhaps, a dormant form²⁹. It should also be emphasised that as yet there is no direct evidence for any role of the 2-5A synthetase and protein kinase in the establishment of the antiviral response. The induction of these enzymes by interferon, therefore, may or may not be essential for the inhibitory process in virus replication in the interferon-treated and virus-infected cell. Whatever the situation, a function of 2-5A synthetase in interferon-treated EC cells remains to be established.

As interferon interacts with EC cells without causing any antiviral or anticellular effects, a comparison between EC cells and their differentiated counterparts may allow the distinction between those interferon-induced events essential for antiviral activity, and other consequences of interferon treatment. A correlation between antiviral and anticellular effects and enhanced kinase activity is confirmed in the present study. This interferon inducibility of the kinase may, in addition, provide a useful marker for distinguishing EC cells from differentiated cells in developmental studies using teratocarcinoma³⁰.

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Table 2 Antiviral and anticellular effects of interferon

Cell type	Antiviral effects (log ₁₀ pfu ml ⁻¹)		Anticellular effects (cell number × 10 ⁵)		
	Control	Interferon-treated	Control	Interferon-treated 250 U ml ⁻¹	Interferon-treated 3,000 U ml ⁻¹
EC cells					
PCC3/A/1	2.1	2.3	21.2	23.3	21.0
PCC4AzaR1	3.0	3.0	61.4	65.0	70.0
Differentiated cells					
PCC3/A/1-D-1 M	2.4	>5.0	50.4	31.0	29.4
L929	2.4	>5.0	13.9	11.8	5.8

The antiviral state of control and interferon-treated cultures was compared by challenging cells with serial twofold dilutions of VSV and determining the concentration of the virus which could kill 50% of the challenged cells after 36 h. Tests were carried out in quadruplicate, using Nunclon 96-well microplaques containing about 30,000 cells per well. The figures represent the plaque-forming units (PFU) per ml of VSV causing 50% cytotoxicity on microscopic examination. These tests were carried out on the same cell batches used for synthetase and kinase assays (Table 1). Anticellular effects were measured on cells incubated with 250 or 3,000 U ml⁻¹ interferon. Cells were plated out at a density of $1-2 \times 10^5$ cells per well in Linbro (6 × 4) 16-mm tissue culture dishes in the presence or absence of interferon. Cell numbers were counted daily for 3 d in quadruplicate treated and control cultures. The figures shown represent cell numbers per well at day 3, when the anticellular effects of interferon were most pronounced.

Prostaglandin D₂ controls pulmonary metastasis of malignant melanoma cells

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Selective metastasis of malignant cells in various neoplastic processes is poorly understood. Metastasis seems to be associated with inherent biochemical and biological properties of tumour cells which guarantee their survival and multiplication. These properties may include surface enzymes¹, interaction with various host defence systems and cells²⁻⁴, combination with host

platelets⁵, or with the host endothelial cells, and adhesiveness of the metastatic cell. Fidler^{2,6,7} has developed a series of malignant melanoma cells that are useful for studying tumour metastasis. By comparing the moderate metastatic parent line, B16F₁, to the highly metastatic line, B16F₁₀, it is possible to study metastasis selectively. Prostaglandins influence various cell functions, and may be involved in regulation of neoplastic processes⁸⁻¹⁰. We have recently reported that prostaglandin D₂ (PGD₂) formation by malignant melanoma cells *in vitro* is inversely correlated with their metastatic potential¹¹. The studies we describe here were designed to determine whether treating the cells with a cyclooxygenase inhibitor, which would block conversion of arachidonic acid to prostaglandins, would affect the metastatic rate of either cell. The results suggest that PGD₂ is involved in the metastasis of these cells.

Monolayers of B16F₁ and B16F₁₀ cells which had reached confluency (in 75-cm² plastic tissue culture flasks, grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum) were incubated for 2 h with 20 µg ml⁻¹ of indomethacin, an irreversible fatty acid cyclooxygenase inhibitor¹². Growth medium containing indomethacin was removed from the monolayers. Cells were then rinsed twice with phosphate buffer saline and then 10 ml per flask of Hank's balance salt solution without magnesium or calcium supplemented with 2% fetal calf serum was added to each flask. Cells were removed

with rubber policemen and pipetted gently to achieve a single cell suspension. Cells were counted, suspended at 2.5×10^5 viable cells per ml and were distributed into plastic tubes, 2 ml per tube. Within 15–20 min of collection, cells were injected intravenously into C57BL/6J mice (Jackson Laboratories). Each mouse received 0.1 ml of cell suspension. Mice were housed in stainless steel cages and were killed 3 weeks later. Lungs were removed, fixed in 3.7% formalin solution, and the number of metastatic foci on each lung was determined using an American Optical stereo zoom microscope.

Injection of B16F₁ and B16F₁₀ malignant melanoma cells into their syngeneic host confirmed that the cells retained their differential metastatic phenotype. B16F₁₀ cells formed 5.1 ± 0.78 metastatic nodules per animal compared to B16F₁ cells which formed 1.7 ± 0.34 metastatic nodules per animal (Table 1). After treatment of the cells with indomethacin, *in vitro*, both B16F₁ and B16F₁₀ metastasis increased noticeably and there was a normalisation of metastatic differences between the two cells: B16F₁₀ = 8.9 ± 2.8 and B16F₁ = 9.9 ± 4.1 (Table 1).

To extend these observations, the previous experiment was repeated. In this case, after cells were collected and suspended at 2.5×10^5 cells per ml in Hank's balance salt solution without magnesium or calcium, one tube received PGD₂ at a concentration that would give 2 mg kg⁻¹ PGD₂ when 0.1 ml was injected intravenously. Cells again were immediately injected into mice intravenously and 3 weeks later the number of metastatic foci per lung was determined. Results are presented in Table 2. These results confirmed again that indomethacin treatment increased the metastatic rate of the cells, particularly the less metastatic B16F₁ line. PGD₂ injection reversed, and decreased indomethacin-enhanced metastasis threefold. In these conditions, indomethacin-treated F₁ cells were slightly more metastatic than untreated F₁₀ cells and PGD₂ injected simultaneously with both cells reduced the rate of metastasis. In these studies, the conditions were adjusted to achieve minimum levels of control metastasis so that an increase due to indomethacin treatment and any subsequent reversal by PGD₂ treatment would be more apparent. Although not shown, injection of prostaglandin F_{2α} and E₂ at 2 mg kg⁻¹ did not significantly affect the metastatic rate of either cell.

Prostaglandin D₂ is the principal arachidonic acid metabolite produced by B16F₁ and B16F₁₀ malignant melanoma cells¹¹. We have previously demonstrated that a quantitative difference exists between the ability of B16F₁ and B16F₁₀ cells to metabolise arachidonic acid to PGD₂ and that this *in vitro* difference correlated inversely with their metastatic rate *in vivo*. Results presented here extend these observations and indicate that *in vitro* treatment of these cells with a compound that blocked conversion of arachidonic acid to prostaglandin increased the metastatic rate of the cells *in vivo*. This was particularly true for

Table 1 Pretreatment of B16F₁ and B16F₁₀ malignant melanoma cells with cyclooxygenase inhibitor normalises their metastatic potential

No. of metastatic colonies on lung surface (bilateral)			
a F ₁₀ control*	b F ₁₀ + indomethacin	c F ₁ control	d F ₁ + indomethacin
9	2	2	3
7	3	1	10
2	10	1	12
4	5	3	33
6	23	1	3
5	19	1	4
5	3	3	4
3	6		
Mean ± s.e.m.			
5.1 ± 0.78	8.9 ± 2.8	1.7 ± 0.34	9.9 ± 4.1

B16 melanoma cells were incubated for 2 h at 37 °C with growth media or 20 µg ml⁻¹ indomethacin. They were then rinsed with PBS and suspended at 2.5×10^5 cells ml⁻¹ and 0.1 ml injected i.v. Three weeks later mice were killed, lungs were removed and the number of metastatic lesions per lung counted.

* Using the Fisher's Exact Test, at the $P \leq 0.05$ level: $a < b$; $a > c$; $c < d$.

Table 2 Prostaglandin D₂ reduction of the metastatic potential of B16 malignant melanoma cells

No. of metastatic colonies on lung surface (bilateral)					
a* F ₁₀ control	b F ₁₀ +INDO	c F ₁₀ +INDO+PGD ₂	d F ₁ Control	e F ₁ +INDO	f F ₁ +INDO+PGD ₂
1	11	2	0		1
1	2	1	0	2	2
2	2	0	0	10	0
1	4	2	0	20	4
0	2	1	0	16	0
3	2	0	0	9	2
2	2	1	3	6	10
10	4	1	1	2	2
3	3	0	1	20	15
1	3	2	1	3	
Mean ± s.e.m.					
2.4 ± 0.88	3.5 ± 0.85	1.0 ± 0.25	0.6 ± 0.30	8.8 ± 2.3	3.6 ± 1.6

B16F₁ and B16F₁₀ cells were incubated for 2 h with 20 µg ml⁻¹ indomethacin (INDO), were then rinsed with PBS and resuspended at 2.5×10^5 cells ml⁻¹. To one half of the treated cells PGD₂ was added to give 2 mg kg⁻¹ when 0.1 ml of the cell suspension was injected i.v. Lungs were collected 3 weeks later, were fixed with 3.7% formalin and metastatic foci were counted using a dissecting microscope.

* Using the Fisher's Exact Test, at the $P \leq 0.05$ level: $a > c$ but not b ; $b > c$; $a > d$; $d < e$ and $e > f$.

the B16F₁ cells that were less metastatic but produced the most PGD₂. Indomethacin treated cells were also co-injected with PGD₂. If PGD₂ was controlling the metastatic rate of the malignant melanoma cells, exogenous reconstitution of their PGD₂ levels should reverse the effect of indomethacin. This was found to be the case. Cells that were made more metastatic by indomethacin treatment reverted to a less metastatic form on co-administration with PGD₂.

One mechanism proposed for B16 cellular metastasis is that platelets adhere to the surface of malignant cells, causing emboli which then plug or attach to the endothelial surface in capillary beds⁵.

Prostaglandin D₂ in sufficient amounts inhibits platelet aggregation in several species^{13,14}, and we have verified this effect in C57B1/6J mice. Our results suggest that PGD₂ is directly involved in prevention of pulmonary metastasis, possibly through its influence on the formation of platelet-tumour emboli. Other effects of PGD₂ on cell-cell interactions, cellular membranes and host defence systems may also be involved.

Indomethacin neutralised the ability of these cells to make PGD₂ and increased their metastatic rate. Exogenous restoration of PGD₂ reversed the effect of indomethacin. The consolidated data from *in vitro* experiments¹¹ and these *in vivo* experiments are consistent with a role of PGD₂ in the inhibition of platelet-tumour emboli. These results suggest that PGD₂ is involved directly in control of metastasis of these malignant melanoma cells. It is not presumed, however, that this is the only mechanism controlling the metastatic rate of these cells. Other biochemical and biological properties of the cells may be intimately involved in the metastatic rate. Results do suggest, however, that PGD₂ is one natural, cellular component controlling pulmonary metastasis of malignant melanoma cells.

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Naloxone-reversible effect of opioids on pinocytosis in *Amoeba proteus*

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A characteristic feature of induced pinocytosis in *Amoeba proteus* is the formation of broad channels by invagination of the cell membrane. This process, which requires Ca²⁺, occurs in response to depolarising cations¹⁻⁴. High Ca²⁺ levels reduce pinocytosis induced by cations such as Na⁺ and Tris⁺, whereas pinocytosis induced by K⁺ is less affected by Ca²⁺ (ref. 4). Agents which interfere with the calcium metabolism of the amoeba will therefore either stimulate or inhibit pinocytosis induced by Na⁺ (ref. 5). Among the agents which are supposed to reduce Ca²⁺ influx across cell membranes or otherwise decrease cellular availability of Ca²⁺ are the opiates and opioid peptides⁶⁻⁹, high doses of which have been reported to affect the amoeba¹⁰. Accordingly, Met-enkephalin, morphine and

codeine potentiate the inhibition of pinocytosis caused by Ca²⁺-binding agents and reverse the calcium blockade of pinocytosis mediated by caffeine¹¹. In this report we show that pinocytosis induced by Na⁺ or Tris⁺ is suppressed by β -endorphin, Met-enkephalin and morphine. These effects were abolished or diminished by an opiate receptor antagonist, (-)naloxone, by increasing the Na⁺ concentration, or by addition of Ca²⁺.

We used *Tetrahymena*-fed *Amoeba proteus* which were cultured in a modified Pringsheim medium¹² or in Prescott-James solution¹³. The results were similar irrespective of culture medium. Pinocytosis was induced by removal of the medium and addition of the inducing solution (100 mM NaCl at pH 6.0, 90 mM NaCl plus 10 mM Tris-HCl at pH 7.0, 100 mM Tris-HCl at pH 7.0 or culture media containing 0.1 mg ml⁻¹ cytochrome c, Trasylol or lysozyme). Pinocytosis activity was determined by counting the number of pinocytotic channels using phase-contrast microscopy. Each amoeba was inspected for 30 s and the channels in 40 amoebae were counted during the first 20 min of the pinocytosis cycle. The mean number of channels per amoeba was taken as an index of pinocytosis activity. Further details of the methods for culture of cells and assay of pinocytosis activity are described elsewhere^{3,14}.

Morphine (4–40 nM), Met-enkephalin (0.2–2 nM) or β -endorphin (0.1–20 nM) (which we will describe collectively as opioids, to include both opiates and opioid peptides) decreased Na⁺-induced pinocytosis by up to 75%. Only low doses of the three opioids were effective and so the relationship between the inhibitory effect of opioids and pinocytosis gave rise to U-shaped dose-response curves. Also, pinocytosis induced by cytochrome c, Trasylol or lysozyme was inhibited by the opioids (Table 1). These cationic proteins give rise to pinocytosis which resembles that induced by Na⁺ with respect to Ca²⁺ requirements^{3,4}. Pinocytosis induced by KCl, on the other hand, was not significantly influenced by the opioids. Low concentrations of (-)naloxone reversed the effects of the opioids whereas the same concentrations of (+)naloxone were not antagonistic. (+)Naloxone possessed about 1/10,000th the activity of (-)naloxone (Fig. 1). A similar low potency of (+)naloxone in three mammalian assay systems has been reported¹⁵.

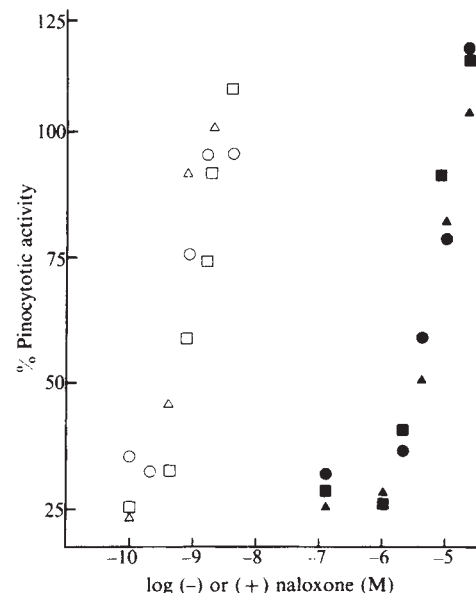


Fig. 1 Reversal by (-)naloxone (open symbols) and (+)naloxone (filled symbols) of the inhibition of pinocytosis due to 8 nM morphine (circles), 0.3 nM β -endorphin (squares) and 0.3 nM Met-enkephalin (triangles). Pinocytosis was induced by 100 mM NaCl, pH 6.0. The pinocytotic activities are given as per cent of the controls. The drugs were added to the inducing salt solution and the experiments were carried out at 23°C. Each experimental point represents a mean value of pinocytosis activity obtained from 40 amoebae during the first 20 min after induction. Note that the first part of the ordinate is deleted.

Table 1 Effect of opioids and naloxone on pinocytosis induced by NaCl (a) and cationic proteins (b)

<i>a</i>	NaCl 100 mM, pH 6.0	Plus 2 nM (-)naloxone	Plus 2 nM (+)naloxone	Plus 20 μM (+)naloxone		
Control	100*	95 ± 1.5 (7)	100 ± 4.0 (7)	99 ± 2.2 (7)		
Morphine 8 nM	34 ± 2.8 (7)	97 ± 3.2 (7)	35 ± 3.7 (7)	77 ± 10.2 (7)		
β-Endorphin 0.3 nM	39 ± 5.1 (7)	90 ± 3.9 (7)	33 ± 3.8 (7)	75 ± 10.4 (7)		
Met-enkephalin 0.3 nM	28 ± 2.2 (7)	95 ± 6.6 (7)	25 ± 2.0 (7)			
<i>b</i>	Cytochrome <i>c</i> 0.1 mg ml ⁻¹	Plus 2 nM (-)naloxone	Lysozyme 0.08 mg ml ⁻¹	Plus 2 nM (-)naloxone	Trasylol 0.1 mg ml ⁻¹	Plus 2 nM (-)naloxone
Control	100*	101 ± 2.9 (3)	100*	97 ± 8.1 (3)	100*	102 ± 3.8 (3)
Morphine 8 nM	55 ± 4.5 (5)	109 ± 5.8 (5)	44 ± 3.6 (5)	93 ± 3.2 (5)	56 ± 6.3 (5)	107 ± 11.3 (5)
β-Endorphin 0.3 nM	52 ± 6.1 (4)	98 ± 2.4 (4)	49 ± 4.4 (4)	95 ± 6.0 (4)	53 ± 4.4 (5)	97 ± 1.5 (5)

(+)Naloxone was synthesised¹⁵ by A. E. Jacobson. Cytochrome c (type VI, Sigma) and Trasylol (Bayer) were dissolved in Prescott-James solution, and lysozyme HCl (Miles-Seravac) was added to the ordinary culture medium—Pringsheim solution.

* Pinocytosis intensity of controls are rated as 100%. Values are $\pm$ s.e.m. of the number of experiments given in parentheses.

The sensitivity of the amoeba to the opioids increased when Na⁺ was replaced by the protonated form of Tris (Tris⁺). This is shown in Fig. 2a, where substitution of Tris⁺ for Na⁺ increased the sensitivity to morphine about 10-fold. (-)Naloxone (0.6–2 nM) antagonised the effect of morphine on both Na⁺- and Tris⁺-induced pinocytosis (Fig. 2b). The results of this and similar experiments suggest that the potency of the antagonist against morphine or Met-enkephalin (Fig. 2d) is increased in the presence of Na⁺. Compared with morphine, Met-enkephalin and β -endorphin were less influenced by Na⁺ (Figs 2c, 3c).

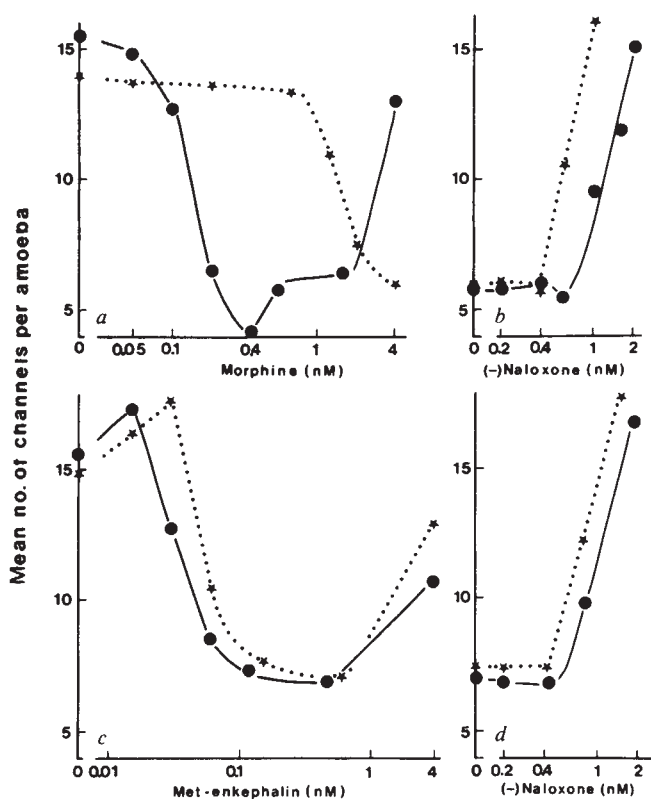


Fig. 2 The effect of morphine and Met-enkephalin on pinocytosis and its reversal by (-)naloxone. Pinocytosis was induced by 100 mM Tris-HCl (●) or 90 mM NaCl plus 10 mM Tris-HCl at pH 7.0 (Na⁺-induced pinocytosis, ★). The conditions for the experiments were as described in Fig. 1 legend. a, The inhibitory effect of morphine chloride (ACO, Solna) on pinocytosis induced by Tris⁺ (●) or Na⁺ (★). b, (-)Naloxone-HCl reverses the effect of 0.6 nM (●) and 4 nM (★) morphine on pinocytosis induced by Tris and sodium, respectively. The cells were from the cell culture used in the experiments of (a). c, The effect of Met-enkephalin on pinocytosis induced by Tris⁺ (●) or Na⁺ (★). d, Inhibition of pinocytosis by 0.12 nM (●) and 0.15 nM (★) Met-enkephalin (Beckman) is reversed by (-)naloxone. The same cell culture as (c).

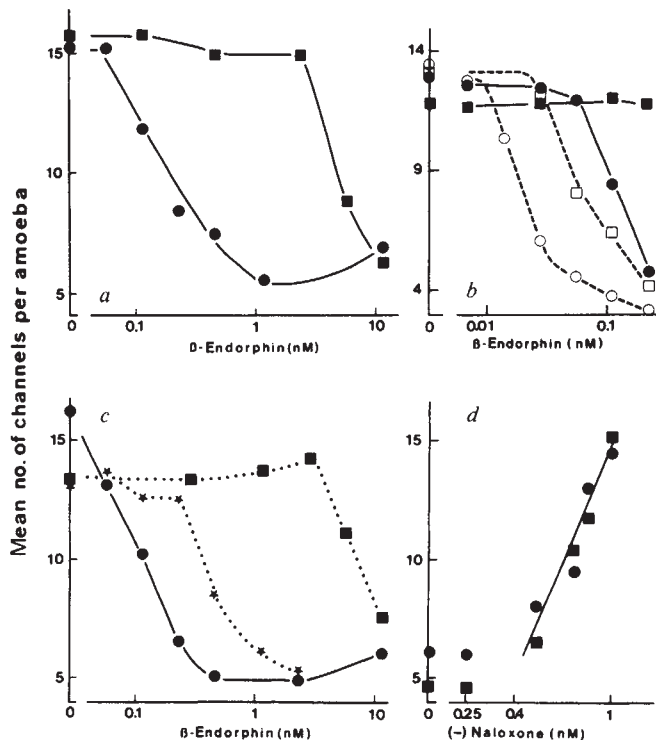


Fig. 3 The effect of Ca²⁺, verapamil and (-)naloxone on inhibition of pinocytosis by β -endorphin. The inducing salt solutions were as outlined in Fig. 2 legend. a, Dose-response curves of β -endorphin (Peninsula Laboratories) on pinocytosis induced by 100 mM Tris-HCl at pH 7.0 (●) and by Tris⁺ plus 10 μ M CaCl₂ (■). b, Inhibition of pinocytosis by β -endorphin is potentiated by verapamil. Pinocytosis was induced by Tris⁺ (circles) or Tris⁺ plus 10 μ M CaCl₂ (squares). Open symbols denote presence of 8×10^{-7} M verapamil. c, Comparison between the inhibitory effect of β -endorphin on pinocytosis induced by Tris⁺ (●), Na⁺ (★) or Na⁺ plus 10 μ M CaCl₂ (■). d, Reversal of the inhibitory effect of 10 nM β -endorphin by (-)naloxone. Pinocytosis was induced by Tris⁺ (●) or Tris⁺ plus 10 μ M CaCl₂ (■).

An even more significant ionic influence was exerted by Ca²⁺. In the presence of 10 μ M CaCl₂ the inhibitory potency of all three opioids was decreased by approximately 90%. This is shown for β -endorphin in Fig. 3a. On the other hand, verapamil, which inhibits Ca²⁺ influx in many cells¹⁶, potentiated the response to the opioids and abolished the effect of Ca²⁺ (Fig. 3b). Unlike Na⁺, Ca²⁺ did not affect the sensitivity to (-)naloxone (Fig. 3d) but decreased that to the opioids even when Na⁺ was the inducing cation (Fig. 3c).

The low concentrations of opioids which affect pinocytosis in *A. proteus* and the enantiomeric stereospecificity of the antagonistic effect of (-)naloxone demonstrated here strongly suggest that these drugs interact at a receptor level. The finding

that the response to opioids in the amoeba is sensitive to Na^+ , may reflect conformational changes of the receptor molecule, similar to those which reduce binding of agonists to opiate receptors in nervous tissue^{17,18}. Furthermore, the difference in Na^+ sensitivity between Met-enkephalin and morphine in binding to mammalian opiate receptors¹⁹ and inhibition of pinocytosis is similar. The opioids may alter the pinocytotic activity either by reducing the intracellular Ca^{2+} or by decreasing the sensitivity to this ion. Their mode of action may, in fact, be similar to that in synaptosomes, where they are reported to inhibit Ca^{2+} influx^{8,20}. However, a physiological function for the opiate receptors in, for example, calcium regulation is difficult to conceive unless ligands for them are discovered in the amoeba itself or in its ecosystem.

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Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurones

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The hypothesis that trophic substances are involved in the normal development and maintenance of the autonomic nervous system has gained much support from studies on the function of nerve growth factor (NGF). *In vitro* at least, both avian and mammalian sensory and sympathetic neurones have a requirement for NGF¹, or for similar, as yet uncharacterised, molecules from homologous non-neuronal cells², glial cells³, glioma cells⁴ or homogenates of certain tissues⁵. Although molecules other than NGF can apparently support the *in vitro* survival of apparently NGF-sensitive neurones^{4,5}, there has been no definitive evidence—excluding preliminary results with parasympathetic neurones of the ciliary ganglion^{6–8}—that other specific trophic factors are essential for neurones insensitive to

NGF. I report here that adult rat brain astrocytes can promote the *in vitro* survival of not only NGF-dependent neurones, such as rat embryo superior cervical ganglion (SCG) cells, but also NGF-insensitive neurones such as the sensory neurones of the rat or chick embryo nodose ganglion (NG). The support of the NGF-dependent neurones is mediated by astrocyte-derived NGF, whereas the survival of NGF-insensitive neurones is brought about by a diffusible factor quite distinct from NGF, but as yet uncharacterised. Evidence is also presented which indicates that the astrocytes can support two distinct populations of neurones in the chick dorsal root ganglion (DRG). It is suggested that these populations represent respectively the previously described small, late-developing, medio-dorsal, NGF-dependent neurones and the early developing, large, ventro-lateral, NGF-insensitive neurones⁹.

Astrocytic glial cells, designated RG18, derived from normal adult rat brain after kainate lesioning of the corpus striatum were maintained as monolayer cultures in 35-mm plastic (Falcon) tissue culture dishes, as previously described¹⁰. Experimental RG18 were seeded (5×10^3 cells per dish) to give almost confluent monolayers at the start of neuronal-astrocyte co-culture. Rat SCG and NG were from 17–19-d-old embryos, and chick DRG and NG were from 8–9-d-old embryos. Rat ganglia were dissected free of connective tissue, washed twice with Eagle's minimal essential medium (MEM), teased into small chunks and digested with 0.25% trypsin in MEM for 30 min at 37°C. Chick embryo ganglia required less vigorous trypsinisation—10 min with 0.1%. Trypsinised ganglia were washed three times with MEM, and dissociated into single-cell suspensions by trituration through a finely drawn siliconised glass Pasteur pipette. Cell suspensions were filtered through 45- μm nylon mesh and counted in a haemocytometer. Dissociated neurones were seeded either directly on to the astrocyte monolayer or the astrocytes were first covered, 3–4 h previously, with 1.0 ml of reconstituted rat tail collagen gel¹¹. The collagen prevents direct contact of neurones and astrocytes but permits free diffusion between them and the medium. The co-culture growth medium (2 ml per dish) was as previously described¹² with the omission of NGF. Control cultures of all neuronal types were grown on collagen gel (1 ml) coated 35-mm dishes in the presence (5 ng per dish) or absence of NGF purified as before⁴. Parallel experiments were carried out in which anti-NGF antiserum (sheep or rabbit antiserum) was included in amounts of 5–100 $\mu\text{g ml}^{-1}$. Cultures were maintained in a 5% CO_2 : 95% air, humidified atmosphere at 36.5°C. Growth medium was replenished every third day and co-cultures were maintained for 3 weeks or more.

As shown in Fig. 1, neurones from all the ganglia tested survived and extended long neurites in co-culture conditions. Very few, if any, neurones survived in control conditions, that is, in the absence of either glial cells or exogenous NGF. A modified Holmes silver stain¹³ procedure was routinely used to identify neuronal processes (Fig. 1a–c), although the large flattened astrocytes are readily distinguished from any neurones even without staining. The survival of SCG and DRG neurones in co-culture could be the result of NGF secretion by the astrocytes. However, this would not explain the survival of nodose ganglion neurones which are insensitive to NGF both in the intact ganglion *in vivo* or *in vitro* (R. Levi-Montalcini, personal communication) and in dissociated culture (R. M. L., unpublished results). Further experiments verified that NGF or an immunologically cross-reactive molecule was being released by the RG18 cells and mediated the survival of the SCG neurones and some of the DRG neurones. Anti-NGF antiserum from two different sources completely abolished the ability of RG18 astrocytes to support the growth of SCG neurones, but only diminished the survival of DRG neurones (Table 1). Increasing the amount of antiserum beyond 5 μg per dish had no further effect on the remaining population of DRG neurones. This clearly suggests that the astrocytes do more than simply support the NGF-sensitive neurones. Nodose ganglion neurones from either chick or rat embryo which survived in

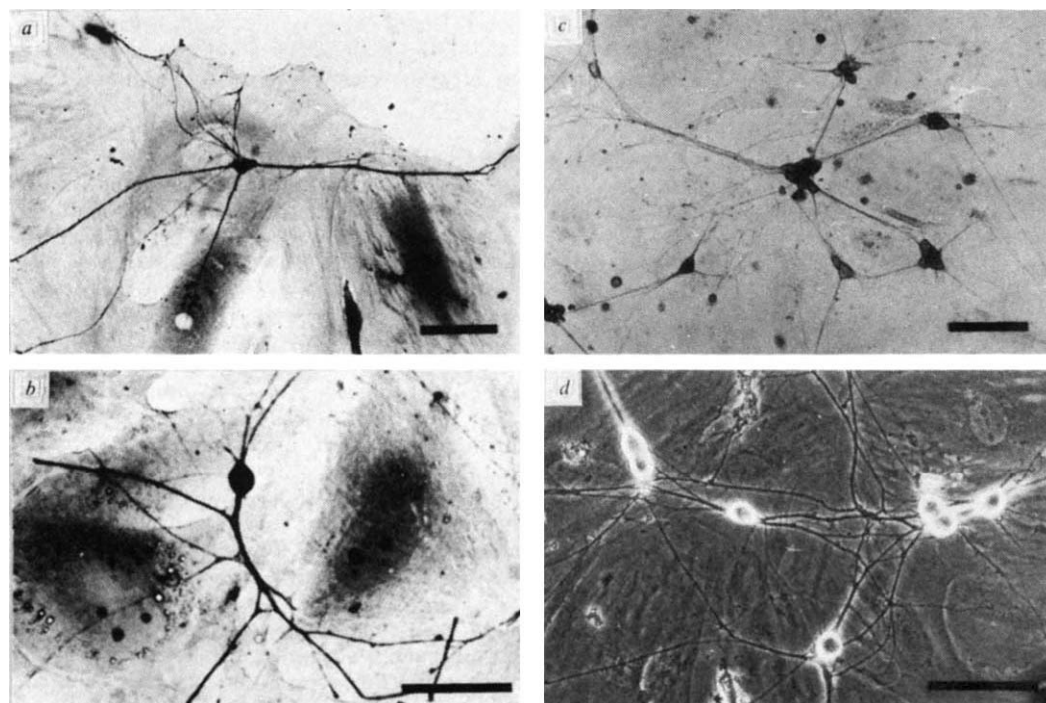


Fig. 1 Dissociated NGF-dependent (SCG, DRG) and NGF-insensitive (NG) neurones surviving in direct contact with adult rat brain astrocytes in the absence of exogenous NGF. Cultures were established as described in the text. *a*, Single rat SCG neurone straddling several RG18 cells, silver stained after 6 d in culture. *b*, Single chick embryo DRG neurone between nuclei of two RG18 cells, silver stained after 6 d *in vitro*. *c*, Network of rat embryo NG neurones on top of astrocyte monolayer, silver stained after 10 d in culture. *d*, Chick embryo NG neurones as seen by phase contrast, unstained preparation after 2 weeks in culture. Calibration bar, 100 μ m. Similar observations are obtained when collagen gel is used, but the neuronal fibres extend in three dimensions, making photographic representation difficult.

co-culture with the RG18 cells were completely unaffected by NGF antiserum, as might be anticipated from their insensitivity to NGF *per se*.

To ascertain that the effect of the astrocytes on neuronal survival was not simply the result of a contact phenomenon, co-cultures were established, as described earlier, in which collagen gel was interspaced between the glial monolayer and the neurones seeded on top. Neuronal survival was generally greater when collagen was used (Table 1) than when neurones and glial cells were in direct contact. The gel has no obvious effect on the growth of either cell type.

The number of neurones seeded per dish in these experiments (Table 1) may seem low relative to other studies. The low seeding was initially the result of low yields of nodose ganglion cells after dissociation, but it was later found that low seedings gave more reproducible and more easily quantitated determinations of neuronal survival. However, the low values do not represent a limitation of the astrocytes to support only a few cells, as an increase in the number of neurones seeded gives a proportional increase in neuronal survival (Table 2). The capacity of the astrocyte cultures in this respect has not been fully determined.

Table 1 Influence of adult rat brain astrocytes on the *in vitro* survival of sensory and sympathetic neurones

Growth surface for dissociated neurones	Exogenous NGF (ng per dish)	Anti-NGF antiserum (μ g per dish)	Neuronal survival (per 35-mm Petri dish)			
			Rat		Chick	
			SCG	NG	DRG	NG
Collagen (1 ml gel)	—	—	2 $\pm$ 1	11 $\pm$ 2	13 $\pm$ 3	10 $\pm$ 5
Collagen	5	—	270 $\pm$ 27	6 $\pm$ 1	208 $\pm$ 42	12 $\pm$ 3
Collagen	5	100*	2 $\pm$ 1	10 $\pm$ 4	14 $\pm$ 1	10 $\pm$ 5
Astrocytes	—	—	341 $\pm$ 5	108 $\pm$ 13	390 $\pm$ 16	416 $\pm$ 67
Astrocytes + collagen	—	—	348 $\pm$ 25	152 $\pm$ 4	818 $\pm$ 95	307 $\pm$ 20
Astrocytes + collagen	—	5*	22 $\pm$ 4	ND	246 $\pm$ 23	ND
Astrocytes + collagen	—	50*	18 $\pm$ 17	ND	198 $\pm$ 19	ND
Astrocytes + collagen	—	100*	16 $\pm$ 4	141 $\pm$ 8	213 $\pm$ 20	310 $\pm$ 18
Astrocytes + collagen	—	100†	10 $\pm$ 5	163 $\pm$ 13	259 $\pm$ 33	288 $\pm$ 24
Astrocytes + collagen	5	—	813 $\pm$ 68	153 $\pm$ 21	1,183 $\pm$ 93	313 $\pm$ 40

Cultures were seeded with either 5,000 superior cervical (SCG) or dorsal root ganglion (DRG) cells or 1,000 nodose ganglion (NG) cells per 35-mm dish, prepared as described in the text. Neuronal survival was determined after 6 d in culture, by counting in the phase contrast microscope all neurones with long processes, scanned for in 25% of the culture dish surface. In this typical experiment values represent the mean $\pm$ s.e.m. of triplicate dishes, evaluated by two independent counters. NGF was purified and assayed for maximal activity as before⁴. Sheep anti-NGF antiserum at 100 ng ml⁻¹ was sufficient to block the activity of 1 ng ml⁻¹ NGF. Rabbit anti-NGF antiserum was similarly active, and both antisera were obtained with 2.5S mouse salivary gland NGF. Astrocytes were seeded 1 week before the experiment and coated with collagen gel 3–4 h before neurones were seeded. Not more than 3 h elapsed between the removal of ganglia and the seeding of neurones. Growth medium—MEM, KCl, glucose, uridine, fluorodeoxyuridine and human placental serum¹²—was prepared freshly at each medium change. Human placental serum was obtained from a local hospital and inactivated 30 min at 56 °C before use. The presence or absence of fluorodeoxyuridine did not affect the results. ND, Not determined.

* Sheep antiserum; † rabbit antiserum.

Table 2 Increase in survival of nodose ganglion (NG) neurones with increase in number seeded

Growth surface	Ganglion cells seeded ($\times 10^{-3}$)	Neuronal survival (per 35-mm dish)	
		Rat NG	Chick NG
Collagen	5	8	24
Collagen + NGF (5 ng)	5	31	72
Astrocytes + collagen	5	728	664
Astrocytes + collagen	10	928	736
Astrocytes + collagen	20	1,912	1,624
Astrocytes + collagen	30	4,328	3,104
Astrocytes + collagen	40	5,504	5,752

Cultures were established as described in the text and Table 1 legend. Neuronal survival was determined after 5 d in culture. Values are for single samples only.

The present findings are consistent with the notion that there are molecules analogous to NGF which are essential for the growth and survival of NGF-insensitive neurones. It also supports the role of glial cells in glial-neuronal interactions, and confirms reports that cells of glial origin (glioma¹⁴, glial¹⁵) are capable of synthesising functionally active NGF. It has previously been shown⁹ that not all cells of the DRG are sensitive to NGF and at least two distinct populations of neurones have been identified in the ganglion by this and other criteria. The present results indicate that astrocytes are capable of promoting survival of not only the NGF-dependent neurones (the small medio-dorsal cells) but also some NGF-insensitive neurones. This is borne out by the observation that anti-NGF antiserum can diminish but is unable to abolish the ability of astrocytes to support DRG neurones in co-culture (Table 1). The developmental similarity between the large neurones of the nodose ganglion and the large, ventro-lateral, NGF-insensitive neurones of the DRG suggests that these latter neurones survive in astrocyte co-culture and that it is their survival which is unaffected by anti-NGF antiserum. Verification of this latter point by biochemical and immunohistological analyses of the surviving cells in different conditions is in progress.

It will be interesting to determine through cell selection procedures whether a single astrocyte from the RG18 cultures is capable of synthesising more than one neuronal growth factor, or whether the ability to synthesise NGF and the ability to support the survival of NGF-insensitive neurones reside in different glial cell types. It will also be interesting to establish if there is any connection between the results of this study and previous reports of non-NGF neuronal growth-promoting activity in glioma cultures⁴ or homogenates of certain tissues⁵.

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Onset of neural function in the lateral line

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The development of the nervous system includes the formation of specific neuronal connections. Some insight into the mechanisms by which these connections are made may be obtained by determining the order in which the elements of a developing system begin to function and examining any changes that may occur in the system shortly after it begins functioning. I have investigated the lateral line of the South African clawed frog, *Xenopus laevis* and I have classified the afferent system into three elements. First, the transduction mechanism, which includes all components that couple the water vibrations to the release of transmitter from the hair cell. Second, the afferent synapse, at which there is transmitter release and subsequent postsynaptic conductance changes. And third, the afferent axon. I report here experiments suggesting that these elements become functional in the following order: first, the axon can conduct action potentials; second, transmission is established; third, the transduction mechanism can modulate transmitter release. This same sequence has been reported in a system related to the lateral line, the auditory pathway of mammals¹.

The peripheral lateral line basically consists of many clusters (neuromasts) of hair cells and a nerve connecting these sensory cells to the brain². Two afferent nerve fibres innervate a neuromast; each connects with half of the hair cells, all of which are orientated in the same direction and opposite to those synapsing on to the other fibre. The hairs project into the water environment and sense aqueous vibrations. In addition, there are efferent fibres which make inhibitory synapses on to the hair cells³. At the earliest developmental time (26 days stage 34 (ref. 4)) when electrical activity in the lateral-line nerves of *Xenopus* tadpoles has been examined it has been essentially adult in character⁵, indicating that the developmental events mentioned above are completed by that time. The experiments reported here were carried out on *Xenopus* tadpoles between the ages of 2 days 8 h (NF 38) and 6 days (NF 48) after fertilisation of the egg (timing accurate to within 2 h). The animal was immersed in saline (3 mM KCl, 10 mM CaCl₂, 125 mM NaCl, 5 mM Tris-HCl, pH 7.3-7.4), anaesthetised by chilling to 1°C,

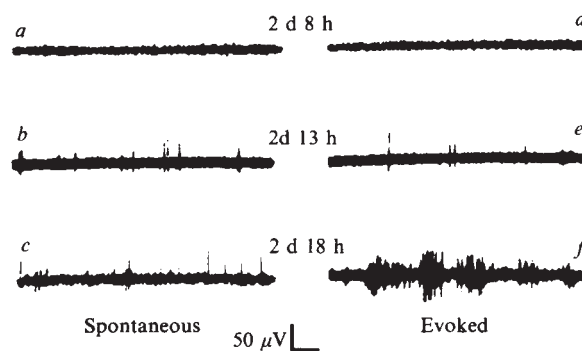


Fig. 1 Electrical activity recorded from lateral-line nerve at three ages. *a-c*, No stimulus; *d-f*, gross stimulation. *a, d*, 2 d 8-h-old animal. *b, e*, 2 d 13-h-old animal. *c, f*, 2 d 18-h-old animal. Records from three different animals. Of five animals recorded from at 2 d 13 h exhibiting spontaneous activity, none had evoked activity. Of five animals recorded from at 2 d 18 h, all had both spontaneous and evoked activity. Horizontal scale, *a, c, d, f*, 0.1 s; *b, e*, 0.5 s; *e*, 0.2 s.

immobilised, the spinal cord transected and the brain removed. Nomarski optics were used to visualise the lateral-line nerve, the neuromasts and individual hairs (kinocilia). Removing a window of skin allowed a recording suction electrode to be applied to the post-orbital lateral-line nerve, either to its cut end or *en passant*, near its entry to the brain. Electrical activity in the nerve was recorded using standard electrophysiological techniques. Three modes of stimulation were used: (1) gross stimulation, a gentle rocking of the preparation table which caused the saline to flow back and forth across the tadpole, exciting many neuromasts; (2) a mechanical stimulation applied to single neuromasts, as described below; and (3) direct electrical stimulation of the nerve by passing current pulses through a stimulating suction electrode. All experiments were done at room temperature (20–22 °C).

The onset of sensory transmission by the afferent nerve was determined by recording from animals of different ages (Fig. 1). At 2 days 8 h, neither spontaneous nor evoked activity was seen, even though the nerve was capable of conducting impulses in response to electrical stimulation (Fig. 2).

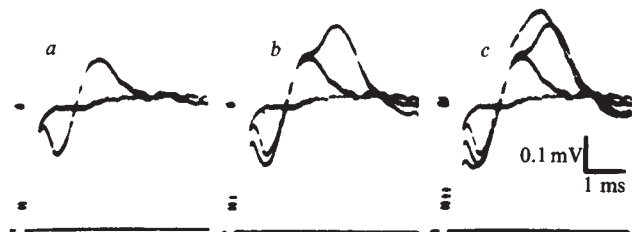


Fig. 2 Direct electrical stimulation of lateral-line nerve in 2 d 8-h-old animal with four current pulses, one every 15 s, delivered by a stimulating suction electrode. Top traces, evoked compound action potentials. Bottom traces, stimulating pulses. *a*, First two trials, superimposed; *b*, first three trials; *c*, all four trials. The fast and slow components, most easily seen in *b*, may correspond to afferent and efferent fibres, respectively.

The appearance of spontaneous activity at 2½ days (Fig. 1*b, e*) suggested that transmission begins at that time. The chemical sensitivity of the synapse offers a method of determining the origin of the spontaneous activity and testing this point. Chemicals suspected of being the afferent transmitter, L-glutamate⁶ (L-Glu) and L-γ-aminobutyric acid⁷ (GABA), were introduced into the saline to see if either would diffuse to the junction and stop activity by desensitising the postsynaptic receptors^{8,9}. The efferent synapse on to the hair cell is likely to be cholinergic¹⁰ and would not be affected by these substances. Exposure to 1 mM L-Glu reversibly abolished spontaneous and evoked activity in animals old enough to exhibit evoked activity (Fig. 3). Spontaneous activity in younger animals was similarly eliminated. The activity usually increased shortly after introduction of L-Glu and only after several minutes fell to zero (data not shown). This pattern of activity, characteristic of desensitisation⁶, was not seen when equal concentrations of GABA or a control, D-Glu, were substituted for L-Glu. The diffusion path is not known but may include passage through holes made in the skin during dissection. These results not only suggest that the afferent transmitter is L-Glu, but also indicate that the spontaneous activity arises at the junction. This interpretation is consistent with the proposal of Harris and Flock² that the spontaneous activity in adult *Xenopus* lateral line is caused by transmitter release and not a pacemaker potential. It remains to be determined whether the final component of transmission is the release of transmitter, the apposition of the pre- and post-synaptic membranes, or the appearance of a sufficient post-synaptic response.

At 2 days 18 h, gross stimulation evoked a vigorous response (Fig. 1*f*) in contrast to its apparent lack of effect at 2 days 13 h (Fig. 1*e*). The onset of sensory function occurs, therefore, at roughly 2½ days. As gross stimulation does not affect synaptic transmission until this time, I conclude that the transduction mechanism is the last of the three afferent elements to function.

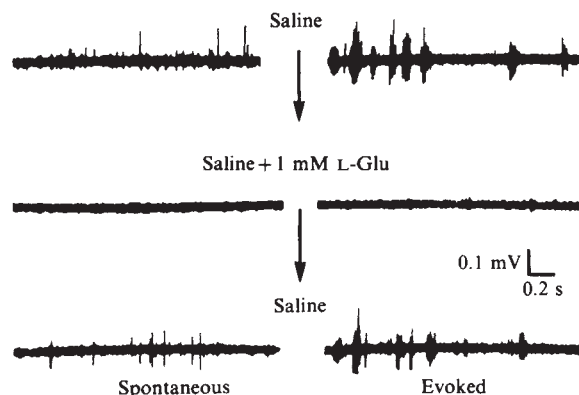


Fig. 3 Glutamate sensitivity of the lateral line in 3 d 15-h-old animal. Left records, no stimulus. Right records, gross stimulation. Top, before addition of L-Glu. Middle, 7 min, after 1 mM L-Glu (pH 7.4) introduced to the bath. Bottom, 9 min after wash and return to normal saline. The L-Glu concentration is comparable to that needed to desensitise Mormyrid lateral line⁶ and may be necessary due to the diffusion barrier surrounding the synapse. Four out of five animals tested before the age of 3 d 10 h showed the response. After that age the system showed decreasing sensitivity to L-Glu, possibly because the diffusion path becomes progressively more impermeable with age.

Developmental changes in the sensory response of the system were studied by means of a mechanical stimulator which provided a one-dimensional controlled movement of 2–20 µm in response to an applied voltage. The sensitivity of single neuromasts was investigated by orientating the stimulator in the direction of maximum sensitivity of the neuromast⁵, placing it in contact with the hairs ~10 µm above the skin surface, and vibrating it to evoke activity from that neuromast. In examination of 50 neuromasts from 18 animals, both units were often seen to respond, one to each phase of the stimulus. The sensitivity of the system increased substantially between 2 days 18 h and 6 days. For example, an equivalent response to 10 cycles of 100 Hz sinusoidal stimulation was elicited at 6 days by a stimulus whose amplitude was only 20% of that required at 2½ days. At the onset of sensory function the system was not able to follow a 200-Hz stimulus, whereas at 6 days, it followed frequencies greater than 350 Hz. The average kinocilium length at 2½ days is ~50% what it is at 6 days.

It seems that the nerve is the first element to function, capable of conducting action potentials before 2 days 8 h after fertilisation. Second, transmission begins at approximately 2½ days. Third, at 2½ days, the transduction mechanism operates, and sensory function begins, approximately 1 day after responses have been found to dimming the illumination of the pineal eye¹¹, and 1 day before the first tectal activity evoked by ocular stimulation with light flashes¹². The sensitivity of the peripheral lateral line increases greatly during the next 4 days. The development of the efferent system has yet to be studied. The efferent axons may be functional before 2 days 8 h. The onset of synaptic inhibition of the hair cells has not yet been determined.

The fact that transmission begins before the transduction mechanism is established implies that this connection is formed without benefit of sensory information. In addition, the first few hours activity carried by the afferent fibres seems to be only spontaneous and unmodulated. It will be interesting to ascertain whether these first connections are altered as the animal develops further.

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Equivalence of Ca^{2+} and Sr^{2+} in transmitter release from K^+ -depolarised nerve terminals

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Depolarisation-secretion coupling at presynaptic nerve terminals is thought to be mediated by the influx of calcium ions from the extracellular fluid through voltage-sensitive channels in the nerve terminal membrane¹. In physiological conditions, the depolarisation is accomplished when the nerve action potential invades the nerve-ending. At the neuromuscular junction, the nerve impulse promotes the synchronous release of many quanta of the transmitter, acetylcholine, the response to which is recorded postsynaptically as the endplate potential (e.p.p.)². Strontium can substitute for Ca^{2+} in the support of transmitter release evoked by nerve impulses^{3–5}, although it is far less effective (on a molar basis) in the generation of the e.p.p. It has been suggested⁵ that Sr^{2+} may be less accessible from the extracellular fluid during the action potential, and might not achieve as high a concentration as Ca^{2+} in the nerve ending so that it would seem less effective. Alternatively, intra-terminal releasing sites may exhibit true selectivity for divalent cations. To help distinguish between these possibilities, I have made use of the long-established observation that when nerve terminals are depolarised by elevating the extracellular potassium ion concentration ($[\text{K}^+]_o$), there is a dramatic increase in the frequency of spontaneous miniature endplate potentials (m.e.p.ps)^{6–9}. This is thought also to be a form of depolarisation-secretion coupling (that is, mediated by Ca^{2+} entry through voltage-gated channels), though of relatively long duration. I have compared the relative abilities of Ca^{2+} and Sr^{2+} in supporting the augmented m.e.p.p. frequency seen in high K^+ Ringer's solutions and report here that they are qualitatively and quantitatively equivalent, in contrast to their differences in the nerve impulse-evoked e.p.p.

All experiments were carried out on the isolated cutaneous pectoris nerve-muscle preparation of the frog (*Rana pipiens*). Conventional electrophysiological techniques were used for intracellular recording of m.e.p.ps from single endplates. Preparations were washed initially in a standard Ringer's solution of the following composition (mM): NaCl 115, KCl 2, NaHCO_3 6, CaCl_2 1.8; pH 6.9–7.2. Before the beginning of an experiment, a preparation was washed for at least 1 h in divalent cation-free Ringer's solution⁵. It was then treated with a Ringer's solution which contained 11 mM KCl and to which were added appropriate amounts of SrCl_2 or CaCl_2 . All solutions contained neostigmine methylsulphate ($1 \mu\text{g ml}^{-1}$), to increase m.e.p.p. amplitudes. In those experiments where the concentration of divalent cation was varied, osmolarity was maintained constant by substitution of 2.5 mM sucrose for every 1 mM SrCl_2 or CaCl_2 (ref. 10). The frequencies of m.e.p.ps were determined in a given solution by recording m.e.p.ps for successive 1-, 2- or 10-s intervals. At least 30 intervals were recorded, from which the mean m.e.p.p. frequency was calculated. Only data from those junctions where the m.e.p.p. frequency in a given solution was relatively stable over time were included in the experimental results.

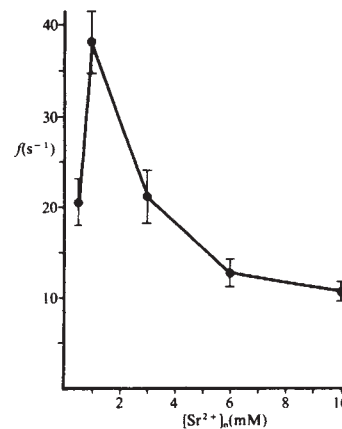


Fig. 1 Effects of $[\text{Sr}^{2+}]_o$ on m.e.p.p. frequency (f) at a single endplate in Ringer's solution containing 11 mM K^+ . Each point represents the mean $\pm$ s.e.m. of 30 1-s counts at a given $[\text{Sr}^{2+}]_o$. The osmolarity was kept constant throughout the experiment by addition of appropriate amounts of sucrose to the Sr^{2+} -Ringer (see text).

Figure 1 shows the results of an experiment in which the effect of external Sr^{2+} concentration ($[\text{Sr}]_o$) on m.e.p.p. frequency in 11 mM K^+ Ringer's solution was studied. In this experiment, the m.e.p.p. frequency increased between 0.5 mM Sr^{2+} and 1.0 mM Sr^{2+} and then declined progressively as the $[\text{Sr}^{2+}]_o$ was raised, up to 10 mM. This non-monotonic dose-response curve is quite similar to that found previously with Ca^{2+} in similar conditions^{8,9,11}. For example, Matthews and Wickelgren found that the relationship between $[\text{Ca}^{2+}]_o$ and m.e.p.p. frequency in high K^+ solutions reached a peak at about 1–3 mM Ca^{2+} and declined at concentrations greater than 3 mM. These authors suggested that the inhibitory effects of high Ca^{2+} are due to its screening of the membrane surface charge, with consequent membrane hyperpolarisation and inactivation of voltage-sensitive Ca^{2+} channels. The qualitatively similar behaviour of Sr^{2+} is consistent with such a screening hypothesis, because Sr^{2+} , as a divalent cation, would also be effective in screening the membrane surface charge and in closing down voltage-gated Ca^{2+} channels^{12,13}. The question remains, however, as to whether the response mediated by Sr^{2+} is quantitatively similar to that of

Table 1 Comparison of the effects of Ca^{2+} and Sr^{2+} on m.e.p.p. frequency in 11 mM K^+ Ringer's solution

Experiment no.	$[\text{Me}^{2+}]$ (mM)	$f_{\text{Ca}} (\text{s}^{-1})$	$f_{\text{Sr}} (\text{s}^{-1})$	$f_{\text{Ca}}/f_{\text{Sr}}$
1	0.5	5.7 ± 0.5	5.2 ± 0.7	1.10
2	0.5	3.9 ± 1.0	4.7 ± 0.9	0.83
3	0.5	6.8 ± 1.9	5.4 ± 1.2	1.26
4	2.0	22.3 ± 3.0	20.9 ± 3.1	1.07
5	2.0	13.8 ± 1.9	13.7 ± 2.4	1.01
6	5.0	13.9 ± 3.0	15.3 ± 3.2	0.91
7	5.0	5.6 ± 1.1	5.7 ± 1.3	0.98
8	5.0	5.2 ± 0.9	5.6 ± 1.1	0.93
9	7.0	13.4 ± 2.0	12.9 ± 1.7	1.04
10	10.0	2.7 ± 0.7	2.4 ± 0.5	1.12
11	10.0	7.7 ± 1.2	8.2 ± 1.2	0.94
Mean = 1.02				

The m.e.p.p. frequency at a given $[\text{Ca}^{2+}]$ (f_{Ca}) was compared at the same endplate with that in equimolar $[\text{Sr}^{2+}]$ (f_{Sr}); $f_{\text{Ca}}/f_{\text{Sr}}$ represents the ratio of the two values, which in no case differs appreciably from unity. Each f value represents the mean $\pm$ s.e.m. of 30 successive counts of 2 s duration in experiments 1, 2, 3, 7, 8, 10 and 11 and of 1 s duration in experiments 4, 5, 6 and 9. For each experiment, $[\text{Me}^{2+}] = [\text{Ca}^{2+}]$ or $[\text{Sr}^{2+}]$. Data are presented from 11 different preparations. Note that although there is considerable variation among preparations, the basic non-monotonic trend with respect to divalent cation concentration is evident. This trend is more clearly shown for Sr^{2+} in Fig. 1 and for Ca^{2+} in ref. 9.

Ca^{2+} , over the concentration range studied in the above experiment. To answer this question, several experiments were carried out in which the m.e.p.p. frequency in high K^+ Ringer's solution containing Ca^{2+} was compared at the same endplate with that in a solution containing equimolar Sr^{2+} . Figure 2 shows a record of such an experiment in which m.e.p.p. frequencies were recorded continuously for approximately 85 min. When the preparation was bathed in a Ringer's solution containing 0.5 mM Ca^{2+} and 2 mM K^+ , the mean m.e.p.p. frequency recorded over about 5 min was $1.2 \pm 0.2 \text{ s}^{-1}$ (mean $\pm$ s.e.m.). When the $[\text{K}^+]_0$ was raised to 11 mM, the m.e.p.p. frequency increased to $24 \pm 3 \text{ s}^{-1}$. When the bathing solution was changed to one containing 0.5 mM Sr^{2+} (in 11 mM K^+), the m.e.p.p. frequency remained at the same level ($23 \pm 2 \text{ s}^{-1}$). On returning to 0.5 mM Ca^{2+} , 11 mM K^+ , the m.e.p.p. frequency was again maintained ($25 \pm 2 \text{ s}^{-1}$). Finally, when $[\text{K}^+]_0$ was reduced again to 2 mM, the m.e.p.p. frequency returned to the control level ($1.4 \pm 0.2 \text{ s}^{-1}$). This experiment suggests that at equimolar concentrations, the two ions are equally capable of supporting the increased m.e.p.p. frequency evoked by high $[\text{K}^+]_0$. Table 1 presents a summary of experiments done on 11 other preparations in which the two ions were compared in concentrations of 0.5–10 mM. It can be seen that, although there is some variation among preparations, there is essentially no difference between Ca^{2+} -activated and Sr^{2+} -activated transmitter release.

The depolarisation of the nerve membrane produced by treatment with high K^+ is a sustained event, as it is determined primarily by the Nernst equilibrium potential for K^+ (ref. 14).

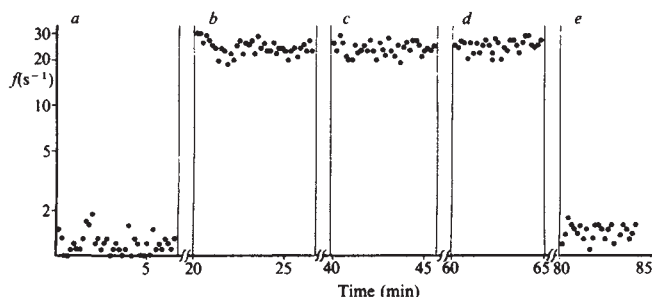


Fig. 2 Augmentation of m.e.p.p. frequency (f) by high $[\text{K}^+]_0$ in Ca^{2+} and Sr^{2+} Ringer's solutions. Continuous recording of m.e.p.p.s over about 85 min. Each point represents the m.e.p.p. frequency over a 10-s counting interval. Note the logarithmic scale on the ordinate axis. An equilibration time of approximately 15 min for each solution change was allowed before data were collected. *a*, The control frequency (in 0.5 mM Ca^{2+} , 2 mM K^+) was $1.2 \pm 0.2 \text{ s}^{-1}$ (mean $\pm$ s.e.m.). *b*, In 0.5 mM Ca^{2+} , 11 mM K^+ , the frequency increased to $24 \pm 3 \text{ s}^{-1}$. *c*, In 0.5 mM Sr^{2+} , 11 mM K^+ , the frequency was $23 \pm 2 \text{ s}^{-1}$. *d*, On returning to 0.5 mM Ca^{2+} , 11 mM K^+ , the frequency remained constant ($25 \pm 2 \text{ s}^{-1}$). *e*, On return to the control solution (0.5 mM Ca^{2+} , 2 mM K^+), the m.e.p.p. frequency returned to $1.4 \pm 0.2 \text{ s}^{-1}$.

This is distinguished from the depolarisation which occurs during the nerve impulse in that the latter is of short duration (of the order of 1 ms) and therefore allows only a transient increase in Ca^{2+} (or Sr^{2+}) permeability. During this brief event, Sr^{2+} might have a lower mobility in the ionic channel and therefore might not enter the terminal and gain access to the intra-terminal releasing sites to the same extent as Ca^{2+} . In contrast, high K^+ provides a prolonged depolarisation to the nerve ending and as Ca^{2+} channels at the presynaptic membrane probably do not exhibit time-dependent inactivation^{15–17}, this depolarisation provides a steady-state, long-lasting increase in Ca^{2+} (or Sr^{2+}) permeability. This prolonged event might allow enough time for Sr^{2+} to accumulate at release sites to the same extent as Ca^{2+} . In these conditions, a difference between the two ions might reflect true intracellular ionic selectivity. The experiments described above, however, indicate that when ion entry factors are minimised (by a prolonged increase in permeability), the differences between Ca^{2+} and Sr^{2+} as supporters of transmitter release

disappear. This suggests that there is divalent cation non-selectivity at intra-terminal releasing sites. Further support for this view comes from a recent report¹⁸ in which transmitter release was studied in neuromuscular preparations treated with tetrodotoxin and 4-aminopyridine to block the nerve action potential. In these conditions, brief electrotonic depolarisation of the nerve ending seems to trigger a regenerative, 'all-or-none' Ca^{2+} current presynaptically¹⁹ and evoke a giant ($>70 \text{ mV}$) e.p.p. postsynaptically. Equimolar substitution of Sr^{2+} for Ca^{2+} gave an e.p.p. of identical amplitude. Thus, even in conditions of brief applied depolarisation, Ca^{2+} and Sr^{2+} seem to be equivalent as supporters of transmitter release, when, as in this case, it is presumed that a maximal ion flux was generated and entry into the nerve terminal ceased to be a limiting factor.

In conclusion, the results of these experiments support the notion that the relatively low efficiency of Sr^{2+} in substituting for Ca^{2+} in nerve impulse-evoked transmitter release does not reflect true intracellular ionic selectivity. Instead, it is probably related to a phenomenon of reduced access of Sr^{2+} to the nerve terminal cytoplasm, because in conditions in which such access ceases to be a limiting factor, the difference in efficiency is abolished.

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Agonist-induced potentiation of acetylcholine sensitivity in denervated skeletal muscle

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The entire surface membrane of denervated skeletal muscle is sensitive to the neuromuscular transmitter, acetylcholine (ACh), whereas in innervated muscle only the junctional area is sensitive. It has been proposed that this difference is due to a 'trophic' effect exerted by ACh in innervated muscle to keep the extrajunctional regions of the surface membrane insensitive to its depolarising action¹. Several studies^{2–4} have demonstrated an agonist-induced potentiation of ACh sensitivity, followed by desensitisation, at the endplate region of normal muscles. The potentiation has been attributed to a cooperative action of ACh on the receptors³. Desensitisation of the extrajunctional regions of denervated muscles by ACh has also been described⁵. We now provide evidence that the transmitter itself potentiates the ACh contracture and depolarisation responses of the denervated muscles of the rat *in vitro* and that it produces this effect by increasing the number of available ACh receptors on the surface membrane.

We used the slow soleus and the fast extensor digitorum longus (EDL) muscles of rats of 100–150 g body weight which had been denervated 4–6 days previously. Muscle contracture and membrane depolarisation responses to diffuse application of ACh were used to test the chemosensitivity of the muscles^{6,7}. The contracture responses to ACh (10^{-6} kg l^{-1}) increased with successive applications of the drug, reaching a maximum after 60–100 min. Figure 1 shows the results from a typical experiment. The maximum increases observed were three to nine times greater than the initial responses to the same dose of ACh ($n=9$). The magnitude of the twitch tension elicited by supramaximal electrical stimulation remained unchanged throughout the experiment. When carbachol was used to elicit contractures the responses again increased with successive applications of the drug. Carbachol is a cholinergic agonist which is not inactivated by cholinesterases; thus, the increases in the response are not due to inactivation of acetylcholinesterase.

The increases in the contracture responses with time of incubation were due to a potentiating action of ACh, as when muscles were incubated in Krebs–Henseleit solution for 100 min before ACh application, they exhibited contracture responses immediately after the preincubation which were comparable in magnitude to the initial responses of muscles which had not been preincubated. Furthermore, the responses of the preincubated muscles increased with successive ACh applications and the time course of the increase was similar to that seen with muscles which had not been preincubated (Fig. 1). As all muscles were stimulated electrically throughout the experiments, including the preincubation period, the ACh potentiation of the contracture responses was not a consequence of muscle contraction.

To determine whether the potentiation of the contracture response was due to an action on the membrane, we measured the depolarisation of the extrajunctional membrane in response to repeated application of ACh (10^{-7} kg l^{-1}) in 4- and 5-d denervated soleus muscles. The depolarisation responses, like the contractures, increased with successive ACh applications, reaching a maximum after 60–100 min (Fig. 2). No significant changes in the resting membrane potential were recorded during the experiments. Values of 65.3 ± 0.8 mV (30 fibres) were

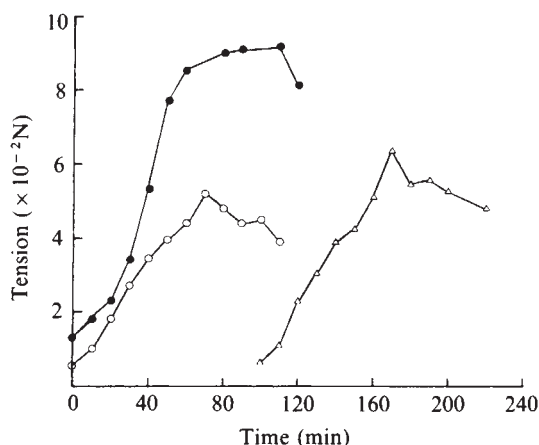


Fig. 1 Potentiation of the ACh contracture responses in denervated muscles. The soleus and EDL muscles of rats denervated 4–6 d previously by unilateral section of the sciatic nerve⁶, were dissected out under ether anaesthesia and suspended in an organ bath containing Krebs–Henseleit solution. All muscles were stimulated electrically at 0.1 Hz and supramaximal intensity throughout the experiment. The initial resting tension was adjusted so that the electrical stimulation produced the maximum contracture. The contractures in response to 10^{-6} kg l^{-1} ACh were recorded as described previously⁶. The drug was removed each time by washing the muscle with 10 bath volumes of Krebs–Henseleit solution. ●, EDL; ○, soleus; △, soleus. In this case ACh application was started at 100 min but electrical stimulation was started at zero time.

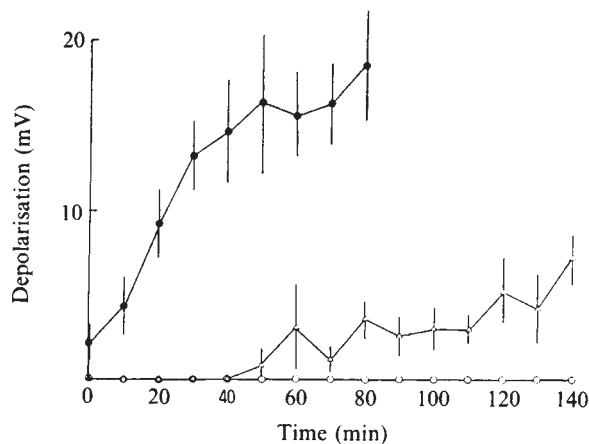


Fig. 2 Effect of ACh on the membrane depolarisation responses of denervated soleus muscles. The resting membrane potential and membrane depolarisation responses to ACh were measured as described previously⁷. Muscles were pinned in an organ bath in Krebs–Henseleit solution and fibres impaled with conventional 3 M KCl-filled micropipettes¹² at extrajunctional regions 2–4 mm from the distal tendons. ACh (10^{-7} kg l^{-1}) was applied diffusely at 10-min intervals, and the drug was removed by washing the muscles with 10 bath applications of Krebs–Henseleit solution. ●, Unblocked muscles ($n=8$); △, muscles blocked with BTX and then treated with ACh (10^{-5} kg l^{-1}) at 10-min intervals ($n=5$); ○, control muscles pretreated with ACh (10^{-7} kg l^{-1}) until no further increases in response were seen (10 applications) and then blocked with BTX and treated again with ACh (10^{-5} kg l^{-1}) at 10-min intervals ($n=5$). Blockade of receptors was effected by incubation of muscles with 0.01 g l^{-1} BTX for 1 h followed by continuous washing with Krebs–Henseleit solution for a further 1 h to remove excess toxin. Values are given as the means with the s.e.m.

recorded before the first application of ACh and 64.3 ± 1.2 mV (30 fibres) after 120–150 min incubation.

To investigate whether the increases in the membrane depolarisation response were due to an increase in the number of receptors available to react with ACh, the effect of the transmitter on muscles blocked with α -bungarotoxin (BTX) was studied. This toxin binds almost irreversibly with ACh receptors⁸. We first established that all available receptors could be blocked, by treating denervated control muscles with ACh until the maximum depolarisation response had been observed, and then incubating the same muscles with the toxin. Such muscles showed no response to ACh subsequently applied at 10-min intervals over 120 min. On the other hand, muscles blocked with BTX in the same conditions but without prior treatment with ACh subsequently exhibited a depolarisation in response to ACh. No responses were seen with the first few applications of the drug but with subsequent applications there was a progressively increasing response (Fig. 2). The new responses were dependent on ACh treatment, as BTX-blocked muscles which were washed for 4 h before ACh applications were insensitive to the first two to four applications of the drug, although small responses were seen with subsequent applications ($n=4$). The new response could be blocked with BTX in the same conditions as before.

Other workers⁹ have demonstrated an increasing contracture response in denervated rat diaphragm blocked with BTX and proposed that BTX alters the membrane architecture, thereby initiating the incorporation of new ACh receptors into the membrane; however, these workers found no effect of ACh on this process. In the present study, the increases in the response in soleus and EDL muscles could be demonstrated regardless of whether or not the muscles had been blocked, and the potentiation was dependent on ACh treatment.

Muscles which have been desensitised by ACh have been reported to bind less BTX than muscles which have not been

treated with ACh¹⁰. Although it is unlikely that agonist-desensitised receptors are present initially in denervated muscles in view of the absence of ACh and the spontaneous reversibility of this type of desensitisation, it is possible that some receptors are initially held in an inactive conformation, unable to bind BTX or ACh, and can be activated by some action of ACh on the muscle membrane. On the other hand, pools of receptors, possibly of a precursor nature and inaccessible to BTX binding, have been demonstrated in other muscles¹¹. Thus, it is possible that the potentiating effect of ACh on muscle chemosensitivity is due to an increase in the number of receptors available to react with ACh or BTX by exposure of previously masked receptors or an incorporation of preformed receptors or receptor precursors into the muscle surface membrane.

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Intracellular pH and the sodium requirement at fertilisation

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Several lines of evidence suggest that ionic messengers are primary agents in the metabolic derepression which occurs at fertilisation. The derepression at fertilisation or parthenogenetic activation of the sea urchin egg occurs in two main phases. The first phase, which triggers the early events of fertilisation, is mediated by transitory increase of intracellular free calcium^{1–3}. The second, which triggers the late events of fertilisation, is mediated by a rise in the intracellular pH (refs 4–6). The transition from the early events of fertilisation of sea urchin eggs to the late events requires a minimal concentration of sodium in the external medium⁷. External Na⁺ is required for the acid efflux which follows fertilisation⁸. Na⁺ requirement and the acid efflux have been correlated in a hypothesis which proposes that internal protons are exchanged for external Na⁺ (refs 8, 9). By using pH-sensitive microelectrodes, we have examined the relationship between external Na⁺ and internal pH more closely. We demonstrate here that the increase of the intracellular pH following egg activation does require external Na⁺. However, the relative insensitivity of the alkalinisation of the egg cytoplasm to large reductions of external Na⁺ is evidence against the Na–H exchange hypothesis.

Intracellular pH was measured using Thomas-type, pH-sensitive microelectrodes with recessed-tips¹⁰. The calibration and use of these electrodes for sea urchin eggs have been described¹¹. As fertilisation by sperm is difficult in the complete absence of sodium¹², we activated the eggs with the calcium ionophore, A23187 (ref. 1). Figure 1 illustrates the change of the intracellular pH of unfertilised eggs of *Lytechinus pictus* exposed to A23187 in natural seawater. The alkalinisation

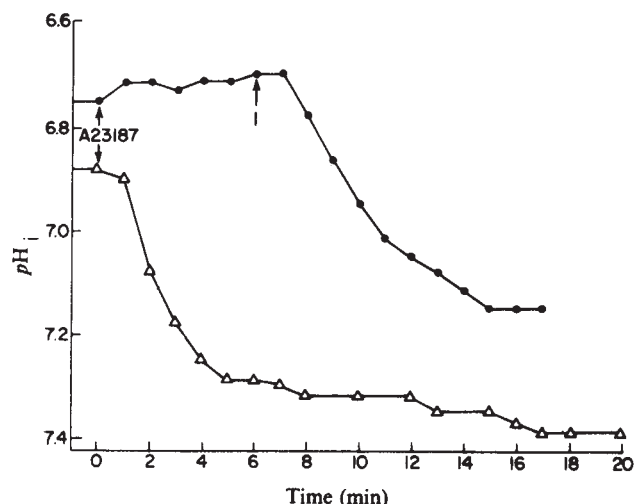


Fig. 1 The external Na⁺ requirement for alkalinisation after egg activation. In all experiments, the *Lytechinus pictus* egg was impaled with two microelectrodes. The egg membrane potential was measured by a conventional microelectrode filled with 3 M KCl. The pH-sensitive microelectrode recorded the membrane potential and a voltage proportional to pH. Both electrode measurements were continuous and recorded on separate channels of a Gould-Brush Mark 220 chart recorder. To obtain the intracellular pH (pH_i), the membrane potential was subtracted from the pH microelectrode potential at intermittent points. Care was taken to assure that any fluctuations of the internal pH was plotted. The noise of the recordings was less than 200 μV. The pH-sensitive electrode was calibrated in 100-mM phosphate buffers (pH 6.5 and 7.5) before and after each experiment. The full response times of these electrodes were 30 s or less. The pH of natural seawater (SW) was adjusted with 1 M HCl or NaOH. Artificial seawater (ASW) had the following composition in mM: NaCl, 480; KCl, 10; CaCl₂, 5.5; MgSO₄, 29; MgCl₂, 27; NaHCO₃, 5. The pH was adjusted with 1 M HCl or NaOH. The temperature was 16–18 °C in all experiments. The activation of eggs by A23187 was in SW, pH 8 (Δ) and in Na-free ASW, pH 8 (●). Na-free ASW was made with choline chloride substitution for NaCl, reduction of KCl to 5 mM and KHCO₃ substitution for NaHCO₃. pH was adjusted with 1 M HCl or KOH. At the arrow, ASW was added to the bath to raise the external Na⁺ to 80 mM.

induced by ionophore activation is similar to that of fertilisation⁶. When eggs were activated by A23187 in Na⁺-free artificial seawater (ASW, choline substitution), the elevation of the fertilisation membrane was observed; however, the rise of the internal pH did not occur (Fig. 1). When external Na⁺ was added back (arrow, Fig. 1), the cytoplasm underwent alkalinisation. Thus, the elevation of cytoplasmic pH associated with activation of the egg required external Na⁺.

It has been reported that amiloride (an inhibitor of passive Na⁺-entry into many Na⁺-transporting epithelia^{13,14}) at 10^{−4} M could block 80% of the acid efflux associated with fertilisation of *Strongylocentrotus purpuratus* eggs when the external Na⁺ was reduced to 50 mM (ref. 8). We tested the effects of low external Na⁺ and the presence of amiloride on the changes of the intracellular pH after fertilisation of *L. pictus* eggs. The addition of 0.5 mM amiloride to 50 mM Na⁺-ASW (choline substitution) did not have any apparent effect on cell division of fertilised eggs. However, inhibitory effects of amiloride in 25 mM Na⁺-ASW were observed. Amiloride at 10^{−4} M in 25 mM Na⁺-ASW did not inhibit the rise of the intracellular pH after fertilisation (Fig. 2). These eggs were seen to divide at a rate similar to control eggs in 25 mM Na⁺-ASW without amiloride. At 2.5–5 × 10^{−4} M, amiloride inhibited the rate of increase in internal pH. These eggs divided 30–60 min after division of control eggs. The delay of cleavage by amiloride between 2.5 and 5 × 10^{−4} M could be eliminated by washes of natural seawater 20 min after fertilisation. At 10^{−3} M, amiloride in 25 mM Na⁺-ASW (glucose substitution, see Fig. 2 legend) blocked the rise of internal pH,

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although the elevation of the fertilisation membrane was observed. The inhibition by amiloride at 1 mM was irreversible, as the internal pH remained acidic after the removal of amiloride by repeated washes of natural seawater (arrow, Fig. 2).

The reduction of external Na^+ to 25 mM (choline substitution) did not inhibit the alkalisation after fertilisation (Fig. 3). An intracellular pH increase in external Na^+ of 5 mM, which is near the minimal external Na^+ required for activation of metabolism after fertilisation⁷, has been observed. The pH of this unfertilised egg was 6.83 and the membrane potential -18 mV. As the pH of the medium was 8, there is a net outward driving force on protons of 50 mV. After fertilisation, the pH of the egg was 7.14 and the membrane potential was -73 mV, giving an inward driving force on protons of 23 mV. This reversal of the driving force on protons with egg activation was similar to observations made in natural seawater⁶. As protons are not in electrochemical equilibrium across the egg plasma membrane, the presence of an active mechanism regulating cytoplasmic pH is implied. It has been reported that transfer of fertilised eggs to Na^+ -free ASW 10 min after fertilisation did not inhibit development⁷. This suggested that external Na^+ was not required for the maintenance of the alkaline pH of fertilised eggs. As seen in Fig. 3, the removal of external Na^+ did not affect the internal pH. Furthermore, we exposed the eggs to a brief pulse of acidic (pH 6.5) Na^+ -free ASW. The cytoplasmic pH fell rapidly and stabilised at a new value of 6.67. During the acidic pulse, the membrane depolarised to -66 mV, due to a loss of newly developed potassium conductance¹⁵. The inward driving force on protons had increased to 76 mV. After the bath was returned to Na^+ -free ASW, pH 8, the cytoplasmic pH and membrane potential recovered. The subsequent elevation of the external Na^+ to 80 mM had little effect on either the membrane potential or intracellular pH. Because, in the absence of external Na^+ , exposure of eggs to acidic medium caused an increase of the inward driving force on protons and the cytoplasmic pH recovered following the return to an alkaline medium, the active mechanism regulating the internal pH of fertilised eggs was not dependent on external Na^+ .

These data demonstrate that external Na^+ is necessary for the rise of the internal pH after egg activation. However, once the

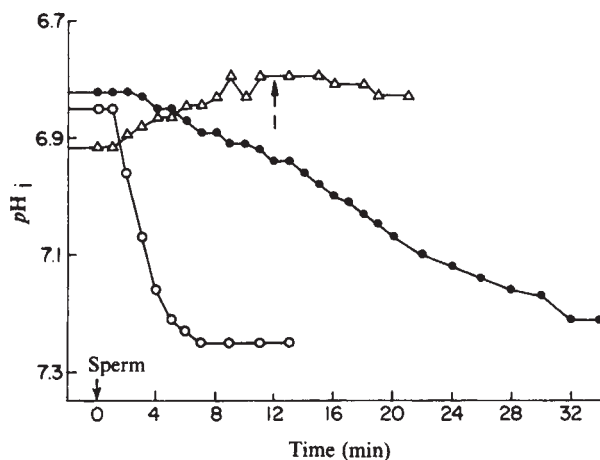


Fig. 2 The effect of amiloride on alkalisation after egg fertilisation. Amiloride at 10^{-4} M (○) had no significant inhibition, at 2.5×10^{-4} M (●) inhibited the rate of alkalisation and at 10^{-3} M (Δ) blocked alkalisation. Amiloride at 1 and 2.5×10^{-4} M was tested in 25 mM Na^+ -ASW, pH 8, where choline was substituted for sodium. Amiloride at 1 mM was tested in 25 mM Na^+ -ASW, pH 8, where glucose (739 mM) was substituted for sodium. No differences in the intracellular pH and membrane potentials during fertilisation of controls were observed between glucose and choline replacement for external sodium. Amiloride was obtained from Merck, Sharp & Dohme Research Laboratory and at 1 mM required extensive stirring and slight heating (35°C), as well as the reduction of the ionic content of ASW. The inhibition by amiloride at 1 mM was not reversed after washing with SW, pH 8 (arrow).

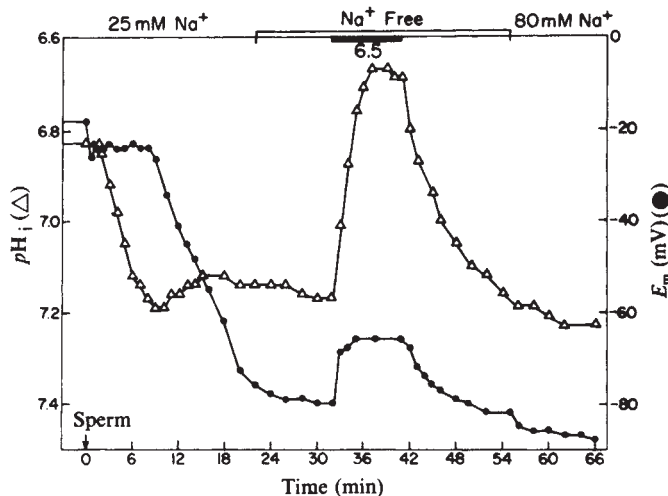


Fig. 3 The effect of external Na^+ and pH on the membrane potential (E_m) (●) and the intracellular pH (pH_i) (Δ) after fertilisation. The egg was fertilised in 25 mM Na^+ -ASW, pH 8 (choline substitution). At 22 min after fertilisation, the external Na^+ was removed. Subsequently, the egg was pulsed for 9 min with Na^+ -free ASW, pH 6.5. At 55 min, ASW was added to the bath to raise the external Na^+ to 80 mM.

rise of the internal pH has been completed, external Na^+ was not required for maintaining the new alkaline pH. As in other tissues, protons are not in electrochemical equilibrium across the cell membrane of the sea urchin egg, implying the existence of an active mechanism regulating the cytoplasmic pH. However, the measured net outward driving force on protons of unfertilised eggs may be a consequence of impalement. Because of the high input impedance of unfertilised sea urchin egg membrane^{15,16} and the large tip diameters of the pH micro-electrode, the low resting potential is probably due to a leakage artefact. Careful recordings of the membrane potentials of unfertilised *L. pictus* eggs with a single KCl microelectrode gave membrane potential values near -75 mV (R.A.S., S.A. McClure and S.S.S., in preparation), in close agreement with the values reported for other species of sea urchin eggs^{16,17}. If the true values of the internal pH and membrane potential of the unfertilised egg are near 6.8 and -75 mV respectively, proton distribution across the plasma membrane would be near passive equilibrium. Alternatively, our reported values of cytoplasmic pH of unfertilised eggs may be a consequence of impalement and may not reflect the true values. This possibility cannot be totally refuted; however, several lines of evidence suggest that the measured values are near the true values despite the lowered membrane potential. Preliminary experiments indicate that the intracellular pH of sea urchin eggs is insensitive to alterations of the membrane potential using voltage clamp techniques and estimation of the internal pH of unfertilised eggs by the distribution of labelled 5,5'-dimethyl-2,4-oxazolidinedione (DMO) gave values near 6.8 (ref. 18). This difficulty of leakage artefact is not encountered with the fertilised egg due to its lower input impedance^{15,16}.

The elevation of the cytoplasmic pH associated with activation of the egg required minimal amounts of external Na^+ , however, the precise mechanism by which a small amount of sodium permits this alkalisation remains unclear. It has been proposed that the external Na^+ is necessary for a Na-H exchange⁸. Our data on the insensitivity of the alkalisation of the egg cytoplasm to reductions of external Na^+ until the critical threshold concentration and the high concentrations of amiloride necessary for inhibition weakens the Na-H exchange hypothesis. A Na-H exchange has been suggested for the regulation of intracellular pH of the mouse soleus muscle fibres. The recovery of the internal pH of the mouse soleus muscle fibres following acidification was significantly slowed by an 11% reduction of external Na^+ and blocked by amiloride at 10^{-4} M

(ref. 19). We have found that the alkalinisation after egg activation was not inhibited by a 99% reduction of external Na^+ and inhibition by amiloride only occurred at concentrations in excess of 10^{-4} M with the external Na^+ reduced to 25 mM. It has been reported that amiloride at 2.5×10^{-4} M in 115 mM Na^+ -ASW did not inhibit acid production after fertilisation of *Psammochinus miliaris* eggs. However, the rate of acid production was slowed in the presence of the drug²⁰. Our data on the relationship between amiloride and external Na^+ concentrations on the alkalinisation of the cytoplasm suggested that sodium and amiloride are competitive for binding sites, similar to amphibian tissues²¹. However, the inhibitory concentrations of amiloride necessary is 1,000-fold greater for eggs than epithelia. The effect of such high concentrations of amiloride on eggs may be nonspecific; that is, the effect may be on some process other than ion exchange. As less than 5 mM external Na^+ is required for the elevation of cytoplasmic pH associated with egg activation and external Na^+ is not required for maintaining the alkaline pH of fertilised eggs, we propose a possible allosteric role for sodium. The binding of sodium to sites on the plasma membrane of the fertilised egg is required for activation of the mechanism directly involved with the alkalinisation of the cytoplasm.

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Membrane response to 1-methyladenine requires the presence of the nucleus

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Meiosis is re-initiated in starfish oocytes by the action of the hormone 1-methyladenine (1-MA)¹. It is thought that the primary trigger is localised at the plasma membrane^{2,3}, and early changes reported are the activation of a Na^+ pump⁴ and variation in membrane potential and conductance^{5,6}. We report here that external application of 1-MA results in an irreversible switching of the starfish oocyte membrane from one stable electrical state to another. This induced change requires the presence of the germinal vesicle.

The resting potential of *Astropecten aurantiacus* oocytes ranges from -70 to -90 mV. Increasing $[\text{K}^+]_{\text{out}}$ by a factor of 10 results in a decrease in the resting potential of 44 mV, approximately as predicted by the Nernst equation for a K^+ selective

membrane. At rest, specific membrane resistance may range from 2 to 8 $\text{k}\Omega \text{ cm}^2$ (egg diameter 300 μm). On addition of 10^{-6} M 1-MA to the bath, we observed a depolarisation of about 0.5 mV min^{-1} and a gradual increase in input resistance of 0.5 $\text{M}\Omega \text{ min}^{-1}$. At 15 to 30 min later the membrane switched to a new stable electrical state of -10 to -20 mV; 50-170 $\text{k}\Omega \text{ cm}^2$. In this state, increasing $[\text{K}^+]_{\text{out}}$ by a factor of 10 did not produce any significant change in the resting potential. Concurrent with the switch, or shortly after, the germinal vesicle was observed to break down. It has been shown elsewhere that sea urchin oocytes have resting potentials in the range -50 to -70 mV, whereas this value in mature eggs is -8 to -16 mV (refs 7, 8). The disappearance of K^+ selectivity after maturation has been reported for amphibians^{9,10} and starfish¹¹.

Offsetting the resting potential of oocytes in the depolarising direction also causes the switch, but the germinal vesicle remains intact. In these cases the switch is reversible (by offsetting in the hyperpolarising direction, or spontaneously). In contrast 1-MA matured oocytes cannot be induced to switch back to the original high potential level. This means the two stable resting states pre-exist in the starfish oocyte, as previously hypothesised¹¹, and that following maturation only one stable state remains. The current-voltage relationships for the starfish oocyte before and after 1-MA treatment are shown in Fig. 1, together with the switch in potential. The voltage excursion in the absence of 1-MA was limited to prevent the switch occurring. The existence of two stable resting states in the untreated oocyte implies an N-shaped *I-V* curve, with two regions of positive slope conductance connected by a third region of negative slope conductance¹¹. Such a curve may only be measured in its entirety in voltage clamp conditions.

We suggest that 1-MA interacts with externally situated plasma membrane structures. This would (1) directly cause the progressive closure of K^+ channels, or (2) cause a leak across the membrane which subsequently depolarises the membrane so closing K^+ channels. In fact, the shape of the curve of the *I-V* relationship for the untreated oocyte (Fig. 1) implies that K^+ channels may be closing due to depolarisation. When the conductance approaches zero the membrane becomes unstable and switches to the low potential state. This resting potential level may be regulated by a different population of ionic channels, which are probably nonspecific. Although the switch can occur either by current injection or by interaction with the hormone, the germinal vesicle only breaks down in the latter

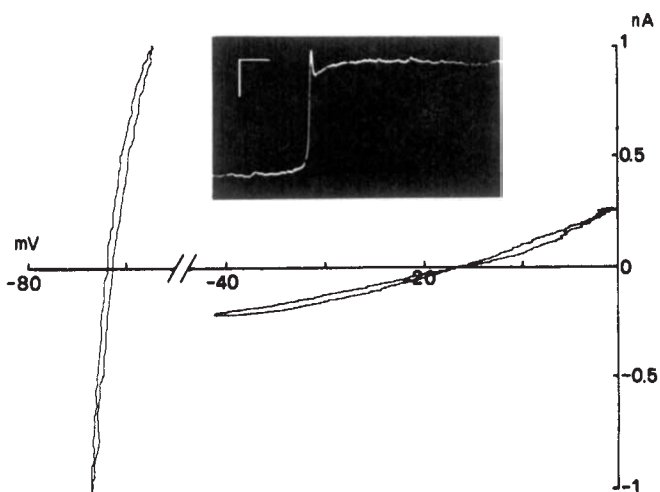


Fig. 1 Recordings from oocytes, complete with jelly and follicle cells, in natural seawater at 20 °C. The inset shows a 1-MA induced switch in an oocyte with an initial resting potential of -80 mV. Horizontal bar represents 5 s, vertical bar 10 mV. The *I-V* curves were obtained by injecting current (*I*), with a sine wave generator (<0.02 Hz), through one intracellular electrode and recording voltage (*V*) with a second intracellular electrode. The curve on the left is from an oocyte before 1-MA treatment, that on the right from the same oocyte after the switch.

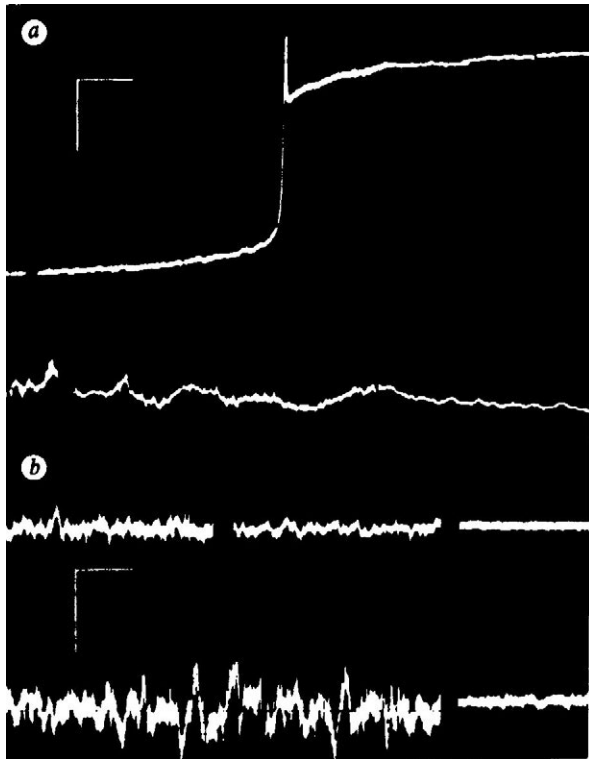


Fig. 2 Anucleated and nucleated fragments were obtained by centrifugation of oocytes in a sucrose gradient at 12,000 r.p.m. or by hand dissection. In each experiment a nucleated and an anucleated fragment of similar size (100–150 μm) were impaled. 1-MA was added to the bath and the responses of both fragments were monitored simultaneously. *a*, The upper trace is from a nucleated fragment which had an original resting potential of -85 mV , and the lower trace is from an anucleated fragment with a resting potential of -95 mV . The horizontal bar represents 5 s, the vertical bar 10 mV. *b*, Voltage noise, a.c. coupled from 1 Hz, in a nucleated fragment (upper) and an anucleated fragment (lower). Upper traces show, from left to right, the spontaneous voltage noise before the switch, after the switch and the microelectrode noise after pulling out the oocyte. Lower traces show, on the left the voltage noise from an anucleated fragment and on the right microelectrode noise after the experiment. The horizontal bar represents 1 s, the vertical bar 0.5 mV.

case. This raises the possibility of the involvement of a secondary factor in the maturation of oocytes.

Starfish oocytes can be divided into nucleate and anucleate halves either manually or by centrifugation. As it is difficult to impale these fragments with a second microelectrode we were unable to study their I - V relationships. However, we were able to monitor their responses to 1-MA. Nucleated fragments behaved as intact oocytes. They had stable resting potentials in the range -70 to -90 mV , and 15 to 30 min after 1-MA addition, the switch occurred and the germinal vesicle broke down (seven fragments, upper trace in Fig. 2*a*). The switch also occurred in these fragments by offsetting the resting potential with the recording electrode. The upper traces in Fig. 2*b* show the voltage noise in a nucleated fragment before and after the 1-MA induced switch. Although in this report we have not analysed voltage noise, this characteristic change following the switch seems to be similar in intact oocytes and nucleated fragments. This difference in voltage noise together with the change in specific membrane resistance (observed in intact oocytes) implies a changed population of functional ionic channels and is consistent with our hypothesis.

Anucleated fragments had resting potentials in the range -20 to -100 mV (seven fragments). In addition these fragments, particularly those with high resting potentials, exhibited large slow fluctuations (lower trace, Fig. 2*a*). Addition of 10^{-6} M 1-MA did not induce the switch in anucleated halves, even after

exposure for 60 min, and in fact offsetting the resting potential with the recording electrode resulted in a linear relationship between injected current and recorded voltage. Anucleated halves with resting potentials in the range -70 to -90 mV had significantly larger peak-to-peak voltage noise than nucleated fragments (Fig. 2*b*). This could be explained by a difference in input resistance; however, nucleated and anucleated fragments selected for recording were approximately the same size (100–150 μm) and of course the two plasma membranes have a common origin.

At present we have no explanation for the large range in resting potential of the anucleated fragments; however, those with lower resting potentials cannot be described as K^+ selective membranes, whilst those with higher potentials were insensitive to changes in $[\text{K}^+]_{\text{out}}$.

It has been suggested^{2,3} that the primary trigger for 1-MA meiosis re-initiation is localised at the plasma membrane. We have shown that although the initial response is at the plasma membrane, the nucleus must be present for this response to occur. It is possible that a secondary factor controlled by, or contained in, the nucleus might be involved in this hormone induced membrane response. However, the recordings from anucleated fragments suggest that removal of the nucleus, in itself, alters the electrical properties of the membrane precluding the 1-MA membrane response.

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Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production

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Although gonadotropin-releasing hormone (GnRH) is believed to mediate the hypothalamic control of pituitary gonadotropin secretion, continuous or repeated administration of GnRH or its agonist analogues has been shown to cause paradoxical anti-fertility effects in several species, including primates^{1–8}. GnRH-induced interruption of reproductive cycles and pregnancy is associated with diminished progesterone production, implying defective function of the corpus luteum^{1,3–6}. These luteolytic effects have been attributed^{4,5} to the well recognised desensitising actions of elevated luteinising hormone (LH) levels on ovarian LH receptors and steroidogenesis^{9,10}, subsequent to GnRH-induced gonadotropin release from the anterior pituitary. However, treatment with high doses of exogenous LH did not cause suppression of serum progesterone levels during early pregnancy in rats⁸, whereas a highly active GnRH analogue was effective in this regard. These observations suggested that GnRH and its agonist analogues, given in high or sustained doses, can exert a direct action on the ovary that is independent

of the pituitary. This hypothesis was further supported by the ability of GnRH and its agonists to inhibit human chorionic gonadotropin (HCG)-induced ovarian and uterine weight gain in hypophysectomised rats¹¹ and to delay the onset of puberty in intact female rats¹². Also, GnRH and its agonist analogues have recently been shown to inhibit steroidogenesis induced by follicle-stimulating hormone (FSH) in cultured granulosa cells, confirming the direct action of such peptides on the ovarian follicle¹³. The marked inhibitory effects of GnRH and its agonists on corpus luteum function suggest that these compounds could exert direct actions by binding to specific receptors on luteal cells. The present experiments, which examine the effects of GnRH agonists on luteal steroidogenesis, demonstrate the existence of such actions and their mediation by specific high-affinity receptor sites present in luteal cell membranes.

Binding studies with the radioactive GnRH analogue ¹²⁵I-[D-Ser(tBu)⁶]des-Gly¹⁰-GnRH *N*-ethylamide (GnRH-A) demonstrated specific uptake of the tracer peptide in both dispersed luteal cells and membrane-rich particles from the luteinised rat ovary. The specific binding of GnRH-A to isolated luteal cells was rapid and saturable, and Scatchard analysis (Fig. 1) indicated that the labelled analogue was bound by a single class of high-affinity receptors ($K_a = 4.4 \times 10^9 \text{ M}^{-1}$) presumably located in the plasma membrane. A particulate membrane-rich fraction from ovarian homogenates also bound the labelled analogue, with a comparably high affinity of $5.8 \times 10^9 \text{ M}^{-1}$. Binding of the labelled analogue was completely inhibited by 10^{-7} M native GnRH. The binding-inhibition curve obtained with unlabelled GnRH was parallel to that derived with the unlabelled analogue, although for any given displacement a

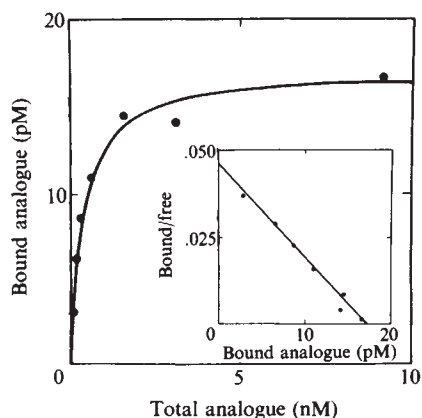


Fig. 1 Binding of ¹²⁵I-labelled GnRH-A to collagenase-dispersed luteal cells prepared from ovaries of 25-d-old female rats treated with 50 IU PMSG and 25 IU HCG to induce ovarian luteinisation⁹. Animals were killed 8 days after PMSG treatment and isolated cells prepared from the minced ovaries by incubation with 2 mg ml⁻¹ of collagenase (Worthington, Type 1 140 U mg⁻¹) in medium 199 containing 0.2% bovine serum albumin (BSA). After 10 min, the collagenase-treated fragments were dispersed by repeated aspiration and filtered through nylon mesh. The resulting cell suspension was preincubated in medium 199/0.2% BSA for 90 min at 36 °C, then centrifuged at 1,500g for 10 min. The cell pellet was resuspended in assay buffer (medium 199/0.1% BSA/25 mM HEPES/0.5 mM bacitracin), and triplicate aliquots of 2×10^6 cells were incubated with $3 \times 10^{-11} \text{ M}$ ¹²⁵I-[D-Ser(tBu)⁶]des-Gly¹⁰-GnRH *N*-ethylamide (1,000 μCi per μg)¹⁵ and increasing concentrations of unlabelled analogue. Incubations were for 80 min at 22 °C in a total volume of 300 μl, and were terminated by dilution with 4 ml of ice-cold phosphate-buffered saline (pH 7.4). The cells were immediately separated by filtration through glass fibre filters (Whatman GF/C) and bound radioactivity was determined by γ spectrometry with a counting efficiency of 60% for ¹²⁵I. Nonspecific binding, assessed in the presence of $3 \times 10^{-7} \text{ M}$ unlabelled GnRH-A, was subtracted from all datum points. The total binding when 50,000 c.p.m. of tracer GnRH-A was added was 4–6%, of which 1/5th represented nonspecific binding. The graph depicts a saturation curve and a Scatchard plot (inset) of the data from a representative experiment. Each point is the mean of triplicate determinations which agreed within 10%. The binding affinity, determined from four experiments by computerised Scatchard analysis¹⁴ was $4.4 \pm 0.75 \times 10^9 \text{ M}^{-1}$, and the binding capacity was $1.5 \times 10^{-11} \text{ M}$ (equivalent to 2,000 sites per cell assuming 75% of cells to be luteal cells).

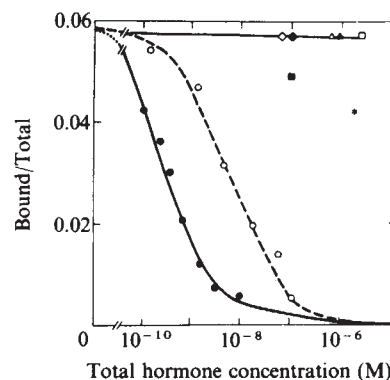


Fig. 2 Specificity of GnRH analogue binding to the 10,000g particulate fraction prepared from PMSG/HCG-primed ovaries as previously described for pituitary tissue¹⁴. The ¹²⁵I-labelled GnRH analogue ($3 \times 10^{-11} \text{ M}$) was incubated with ovarian particles equivalent to 180 μg of membrane protein, with increasing concentrations of unlabelled GnRH-A (●) or native GnRH (○). Incubations were carried out in ice for 80 min in 0.5 ml of assay buffer (10 mM Tris/HCl, pH 7.6, containing 1 mM dithiothreitol and 0.1% BSA). Termination of the incubations and separation of the bound fractions were as described for Fig. 1. The equilibrium association constants (K_a) for the GnRH analogue and native GnRH were $5.8 \pm 1.0 \times 10^9 \text{ M}^{-1}$ ($n = 3$) and $2.5 \times 10^8 \text{ M}^{-1}$, respectively. The binding capacity (receptor concentration) for GnRH-A was $51 \pm 12 \text{ fmol per mg}$ of ovarian membrane protein. Angiotensin I (▲), angiotensin II (△), thyrotropin-releasing hormone (□), somatostatin (*), ovine LH (◆), ovine prolactin (◇) and HCG (■) (CR 119) were diluted in assay buffer and added at the final concentrations shown.

10-fold higher concentration of GnRH was required (Fig. 2). Thus, native GnRH binds to the same sites as the analogue, albeit with significantly lower affinity ($K_a = 2.5 \times 10^8 \text{ M}^{-1}$). Particulate fractions prepared from immature rat ovaries 3 days after priming with pregnant mare serum gonadotropin (PMSG) alone, before the extensive luteinisation induced by HCG treatment, also bound the GnRH analogue with high affinity ($K_a = 9.7 \times 10^9 \text{ M}^{-1}$).

The specificity of binding of the labelled GnRH analogue by ovarian receptor sites was examined by competition studies with a series of peptides, as shown in Fig. 2. Angiotensin I, angiotensin II, thyrotropin-releasing hormone and gonadotropic hormones did not inhibit binding of the analogue to ovarian receptors. Only one peptide tested, somatostatin, was slightly inhibitory at high concentrations (10^{-6} M). The tissue specificity of GnRH binding was indicated by the absence of analogue uptake in similar fractions prepared from liver, kidney, adrenal cortex, hypothalamus and cerebral cortex.

To determine the biological function of the ovarian GnRH receptors, the action of the GnRH analogue on progesterone production was examined in isolated luteal cells. In the presence of $5 \times 10^{-7} \text{ M}$ GnRH-A, basal progesterone production was reduced by 25% and the stimulating effect of low concentrations of HCG ($< 10^{-13} \text{ M}$) was completely inhibited (Fig. 3). In the presence of the GnRH analogue, the maximum progesterone production in response to HCG was similar to that observed in control cells, although a much higher concentration of HCG was required to evoke the maximum steroid response. The ED_{50} for HCG stimulation of progesterone production was increased 25-fold, from 2.5×10^{-13} to $6.0 \times 10^{-12} \text{ M}$, in cells incubated with $5 \times 10^{-7} \text{ M}$ GnRH-A. Inhibitory effects of the GnRH analogue, as well as natural-sequence GnRH, were observed at concentrations as low as 10^{-9} M . The inhibitory effect of GnRH peptides on gonadotropin-induced steroidogenesis was not due to interference with hormone binding, as high concentrations of the analogue (up to 10^{-5} M) had no effect on specific uptake of ¹²⁵I-HCG and ¹²⁵I-labelled human FSH by ovarian homogenates.

These studies have demonstrated the presence of specific receptor sites for GnRH and an agonist analogue in both isolated luteal cells and particulate membrane-rich fractions prepared from luteinised ovaries. The affinities of the ovarian

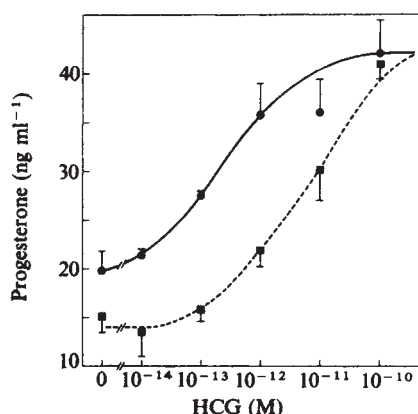


Fig. 3 Effect of [D-Ser(tBu)⁶]des-Gly¹⁰-GnRH N-ethylamide on HCG-induced progesterone production by isolated luteal cells. The cells were prepared as described in Fig. 1 legend, and 2-ml aliquots of cell suspension (2×10^6 cells ml⁻¹) were added to plastic vials containing appropriate dilutions of the hormones. After incubation for 3 h at 36 °C, the suspensions were transferred to glass tubes, centrifuged at 3,000g for 10 min, and the supernatant analysed for progesterone by radioimmunoassay¹⁶. Each point represents the mean $\pm$ s.e.m. of values from triplicate incubations. ●, -GnRH-A; ■, +GnRH-A (5×10^{-7} M).

receptors for both GnRH and the highly active analogue were similar to those of the pituitary GnRH receptors for these peptides¹⁴. However, the GnRH receptor concentration in the ovary was only 20% of that in the pituitary (50 and 235 fmol per mg protein, respectively). Inhibition of progesterone production in isolated luteal cells was observed with low concentrations of both GnRH and its active analogue, consistent with the high binding affinity of the luteal cell receptors. These results suggest that ovarian GnRH receptors mediate the extra-pituitary action of exogenous GnRH and its agonist analogues on progesterone production by the corpus luteum. In addition to their presence in luteinised ovaries, the demonstration of GnRH receptors in ovaries of PMSG-treated rats suggests that such sites mediate the recently reported inhibitory effect of GnRH on FSH-stimulated oestrogen production by granulosa cells¹³.

The present observations in ovarian luteal cells, together with those of Hsueh and Erickson¹³ in granulosa cells, provide an explanation for the paradoxical effects of GnRH and related compounds on reproductive processes in the female. It is clear that the actions of GnRH agonists are mediated through specific receptors that are separate from the gonadotropin receptors in the ovary, and that such sites are present in both granulosa and luteal cells. The inhibitory effects of GnRH and its analogues cannot be ascribed to interference with gonadotropin binding, thus implying that GnRH and its agonists act at a stage after gonadotropic hormone-receptor interaction. In the present study, the major effect of the analogue was to reduce target-cell sensitivity to gonadotropin, causing a shift in the dose-response curve, with no change in the maximum progesterone production evoked by high concentrations of chorionic gonadotropin. These results indicate that the GnRH analogue acts as a competitive inhibitor of gonadotropin-stimulated progesterone production, at least in short-term studies.

Anti-fertility effects of GnRH and its analogues have been previously observed *in vivo* after pharmacological doses of these peptides. Our findings have demonstrated that receptor-mediated inhibitory actions of GnRH and an active analogue are detectable *in vitro* at low concentrations of the peptides. Because GnRH is undetectable in the systemic circulation, it is unlikely that GnRH of hypothalamic origin could reach the ovary in sufficient concentration to exert a direct action on the corpus luteum. It is conceivable that GnRH or a related peptide is produced in the uterus and transported to the ovary, or is formed locally in the ovary where it acts as a mediator of normal luteolysis. This mechanism, and the possibility that GnRH binds to a heterologous receptor in the ovary in a manner analogous to

the morphine/enkephalin interaction, raise intriguing questions about the biological significance of the extra-pituitary actions of GnRH and its analogues.

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Ovarian ecdysone secretion is controlled by a brain hormone in an adult mosquito

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In the mosquito *Aedes aegypti*, a blood meal triggers egg development. Early workers used the simple technique of decapitation, or neck ligation, to show that a factor from the head was required for normal egg development^{1–4}. Lea subsequently demonstrated that the source of this factor was the medial neurosecretory cells of the brain⁵. The hormone from these cells, which he termed the egg development neurosecretory hormone (EDNH), is stored in the corpus cardiacum (CC) and released shortly after a blood meal⁶. Previous work had demonstrated that the ovary begins to produce ecdysone after a blood meal⁸. Considerable evidence suggests that the ecdysone, after being converted to 20-hydroxyecdysone, stimulates the synthesis and secretion of vitellogenin by the fat body^{8–12}. The vitellogenin is taken up by the growing oocytes and becomes part of the yolk. Analysis of the amount of 20-hydroxyecdysone present in females after a blood meal showed that significant quantities were first seen 4–6 h after a blood meal⁸. Vitellogenin synthesis begins at about the same time¹³. The early decapitation experiments of Gillett demonstrated that the head was necessary for 4–8 h after a blood meal for egg development to proceed normally³. A similar critical time was found for the control of vitellogenin synthesis by the brain hormone⁷. The coincidence of this timing suggested to us that the ovary was the target of the brain hormone. The experiments reported here were designed to test the hypothesis that the brain hormone released after a blood meal acted on the ovary, causing the synthesis of ecdysone. Our results confirm this.

In earlier work we had successfully stimulated vitellogenin synthesis by incubating ecdysone-secreting ovaries with fat body preparations⁷. Our success with this *in vitro* approach prompted us to try to activate ovarian secretion of ecdysone by incubating ovaries with brains. Ecdysone in the medium after incubation was measured using an ecdysone radioimmunoassay¹⁴. We found that incubating whole brains (not including the CC, as it is removed with the aorta) with ovaries stimulates ecdysone secretion (Table 1). This effect is almost entirely due to the

Table 1 Effect of parts of the central nervous system on ecdysone secretion by ovaries

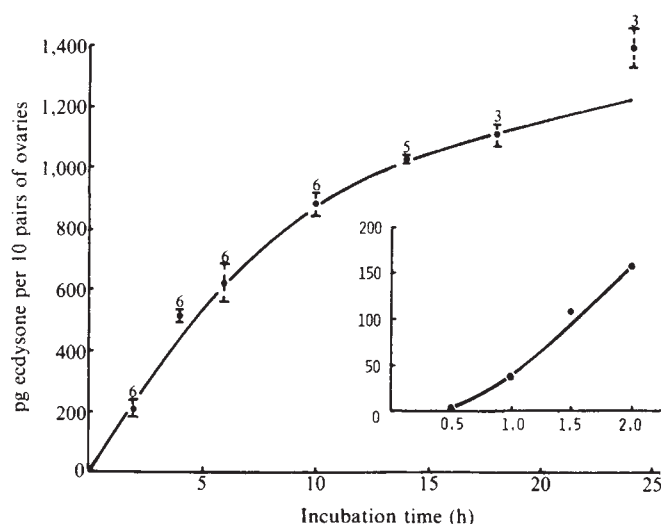
	pg ecdysone per 10 pairs of ovaries $\pm$ s.e.m.
<i>Aedes aegypti</i> (mosquito)	
Adult female	
Whole brain	510 $\pm$ 68
Midbrain	339 $\pm$ 39
Optic lobes	0
Suboesophageal ganglia	16 $\pm$ 10
Thoracic ganglia	18 $\pm$ 0.9
Abdominal nerve cord	46 $\pm$ 6.9
Adult male	
Whole brain	496 $\pm$ 58
4th instar larva	
Whole brain	206 $\pm$ 17
<i>Periplaneta americana</i> (cockroach)	
Larval CC	744 $\pm$ 82
Adult CC	505 $\pm$ 52
<i>Papilio polyxenes</i> (butterfly)	
Larval brain and CC	163 $\pm$ 28
Adult brain and CC	149 $\pm$ 17
<i>Musca domestica</i> (fly)	
Adult brain	132 $\pm$ 18

Parts of the nervous system from five animals were frozen and thawed and incubated with ovaries from 10 female mosquitoes for 18 h at 25 °C in sterile *Aedes* saline¹⁸ containing 0.15 mg ml⁻¹ penicillin and 0.25 mg ml⁻¹ streptomycin. This saline is isosmotic with haemolymph¹⁹. The adults used were 3–5 d old and were maintained on 3% sucrose at 27 °C. The medium and one rinse of the ovaries were combined and assayed for ecdysone using a radioimmunoassay (RIA)¹⁴. As we have previously shown that ovaries secrete only α -ecdysone when incubated *in vitro*⁸, the results are expressed as pg ecdysone per 10 pairs of ovaries. The antiserum used was approximately equally sensitive to α -ecdysone and 20-hydroxyecdysone. Ovaries without any added nervous system gave a background of 40 pg of ecdysone that was subtracted from the above values in this experiment only. Each value represents the mean $\pm$ s.e.m. of three replicate studies. No correction is made in these data for the relative size of the brains used.

midbrain; other parts of the nervous system have little or no effect. Brains from adult males and from larvae also stimulate ovaries, although larval brains have half the activity of adult brains. Surprisingly, the CC and/or brains of several related insects activate ecdysone synthesis by mosquito ovaries (Table 1). The CC of larval cockroaches show considerably greater activity than those of adults. Cockroach CC is also much more active than brains and CC from a butterfly or the brains of a fly. The control of ovarian ecdysone secretion by a brain hormone in *Locusta* has been recently described¹⁵ but in this case male or larval brain and CC were not stimulatory.

Saline extracts of whole mosquito heads also stimulate ecdysone production by the ovary. Ecdysone synthesis is linear for 10 h after exposure to head extracts, with a lag of 0.5 h (Fig. 1). In contrast, there is a delay of 6–8 h after a blood meal before the main peak of ecdysone is seen in the whole animal⁸. As the response *in vitro* is almost immediate (Fig. 1), this suggests that release of brain hormone *in vivo* is delayed. Gillett³ came to the same conclusion by noting the effects of decapitation on egg development. The incubated ovaries are quite sensitive to the factor in head extracts: the amount of hormone present in 1.5–2 heads is sufficient to stimulate a half-maximal response in 10 pairs of ovaries incubated in 50 μ l of extract (Fig. 2). At high concentrations of head extract the stimulation of ecdysone is inhibited. This effect has been noted repeatedly and is apparently due to a detrimental factor in the extract that is diluted out more rapidly than the hormone. Heating the extract has no effect on the inhibition.

Evidence that the ovary produces ecdysone *de novo* in response to the brain hormone was obtained by examining the amount of ecdysone in the ovaries and incubation medium before and after exposure to head extract (Table 2). A small amount of ecdysone is detectable in the ovary before the

**Fig. 1** Time course of ecdysone synthesis in response to head extract. 10 mg of heads were homogenised in 1 ml of sterile *Aedes* saline and treated as described in Table 2 legend except that the extract was not heated. The medium was assayed for ecdysone by RIA¹⁴ after incubation. The data points represent the mean $\pm$ s.e.m. The number of replicates is indicated. The inset represents a separate experiment with two replicates, each with 20 pairs of ovaries. Ovary donors were as described in Table 1.

experiment. After 18 h with the head extract, 30 times more ecdysone is found in the incubation medium than in the ovary.

We have demonstrated that the ovary is indeed the target tissue of a brain hormone that stimulates ecdysone synthesis. This hormone is probably identical with the EDNH described by Lea^{5,6}, as the active factor is found in the region of the brain containing the medial neurosecretory cells (Table 1). A major unsolved problem concerns the nature of the stimuli that cause the release of EDNH after a blood meal. Neither the act of feeding nor stretching of the abdomen during feeding seems to be important⁶. There is some evidence that a humoral agent released after blood feeding is involved; this may be an early product of blood meal digestion¹⁶.

EDNH is analogous in function to PTTH, the prothoracicotrophic hormone found in immature insects. Both cause the synthesis of ecdysone by their respective target tissues; the function of the ecdysone in immature insects is to cause moulting. The similarity may be more than analogous, given that

Table 2 Synthesis of ecdysone by ovaries in response to head extract

	pg ecdysone per 10 pairs of ovaries $\pm$ s.e.m.
Ovaries before incubation	14
Saline extract before incubation	0
Ovaries after incubation	35
Saline extract after incubation	1,100 $\pm$ 25

Heads were obtained from 5–10-d old adult mosquitoes maintained on 3% sucrose by shaking animals frozen at –60 °C to break them apart and separating out the heads using US standard sieves. Heads were retained by a mesh size of 0.425 mm, lyophilised and 5 mg (36 μ g per head) homogenised in 1 ml of sterile *Aedes* saline¹⁸. The extract was then sonified three times for 0.5 min and centrifuged for 5 min at 9,000g. The extract was heated at 50 °C for 15 min and centrifuged again. Heating effects some purification but does not significantly reduce the activity of the extract. Samples of supernatant (50 μ l) were incubated with 3 groups of 10 pairs of ovaries each. After 18 h the ovaries were removed and combined to provide enough material for the RIA. Also 30 pairs of ovaries that were not incubated were dissected. The ovaries were homogenised and extracted with 50% methanol. The supernatant after centrifugation for 5 min at 9,000g was dried down and assayed for ecdysone by RIA¹⁴. The saline extract before and after incubation was also analysed for ecdysone. Ovary donors were as described in Table 1.

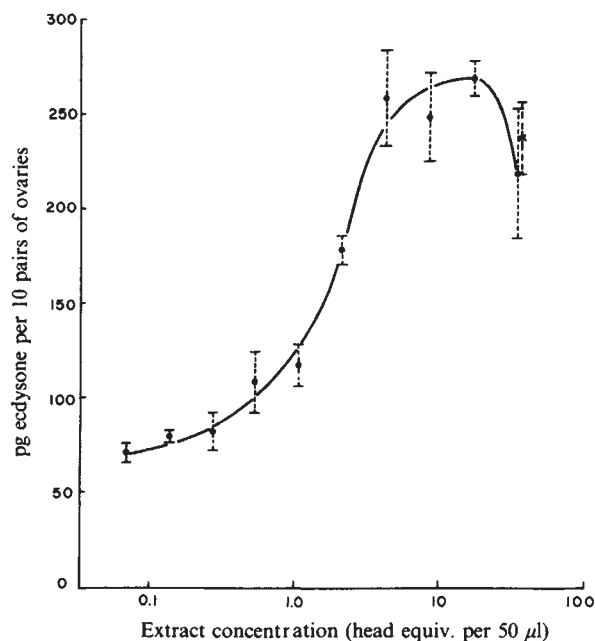


Fig. 2 Ecdysone synthesis by ovaries in response to increasing amounts of head extract. 20 mg of mosquito heads obtained as described in Table 2 legend were homogenised in 0.8 ml of sterile saline¹⁸. The extract was frozen and thawed and then centrifuged for 30 min at 10,000g. The sample was divided, one part was serially diluted and the other heated at 50 °C for 15 min. Ovaries from 10 7-d-old females were incubated with 50-µl samples of the extract for 6 h at 25 °C. Ecdysone was determined in the incubation medium by RIA¹⁴. The results were converted to head equivalents by the factor, 1 head = 36 µg. Data given are mean $\pm$ s.e.m. of five replicates. X Indicates the heat-treated sample.

EDNH activity is present in larval mosquito brains and the brains and CC of several other insects. It is possible that these two hormones are structurally similar and therefore homologous to some degree. Work in our laboratory on the chemistry of EDNH has shown it to be a peptide of molecular weight about 6,500 with some similarities to PTTH from *Bombyx mori*¹⁷.

The neuroendocrine control of ecdysone synthesis by the mosquito ovary resembles in several ways the control of oestrogen synthesis by the vertebrate ovary. In both, a peptide produced by neurosecretory cells or the pituitary stimulates the synthesis of a steroid by the ovary. Perhaps the cyclic production of eggs that occurs in both is best accomplished by this type of control mechanism.

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A circadian oscillator in cultured cells of chicken pineal gland

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The activity of serotonin *N*-acetyltransferase, the key enzyme of melatonin synthesis, shows a marked circadian rhythm in the pineal glands of various animal species¹⁻¹⁰. The regulation mechanism of the *N*-acetyltransferase rhythm in birds is different from that in mammals. *N*-Acetyltransferase activity in rat pineal gland is controlled by the central nervous system through the sympathetic nerves from the superior cervical ganglion¹⁻⁶, while in chicken the endogenous oscillator for *N*-acetyltransferase rhythm is presumably located in the pineal gland. Recently it has been shown that *N*-acetyltransferase activity oscillates in a circadian manner in the organ culture of chicken pineal glands¹¹⁻¹³. When chicken pineal glands were organ-cultured under continuous illumination, the nocturnal increase of enzyme activity was suppressed. These observations suggested that chicken pineal gland contains a circadian oscillator, a photoreceptor and melatonin-synthesising machinery. A central question arises whether the circadian oscillation of *N*-acetyltransferase activity and its response to environmental lighting are generated within the cell or are emergent properties of interaction between different types of pineal cells. I report here that in the dispersed cell culture of chicken pineal gland, *N*-acetyltransferase activity exhibits a circadian rhythm and responds to environmental lighting in the same manner as in the organ culture.

Chickens (White Leghorn cockerels) were raised under the lighting schedule with lights on from 7 a.m. to 7 p.m. for 12-14 days after hatching. After decapitation, their pineal glands were dissociated by treatment with collagenase. More than 90% of the cells were viable as judged by Trypan blue exclusion. Shortly after the treatment with collagenase, most of the cells were dispersed and there was little cell-cell attachment. The typical morphological appearances of the cultured cells are shown in Fig. 1. After inoculation in dishes, some of the cells tended to reaggregate into small clusters. Within 24 h fibroblasts had developed processes. Round cells were alive and mostly attached to fibroblasts, although some remained isolated.

When dispersed cells were cultured in a light-dark cycle (LD 12:12), both the specific activity (per mg protein) and the total activity (per dish) of *N*-acetyltransferase were 5-10-fold higher during the dark periods than in the light periods on days 2 and 3 of culture (Fig. 2a, b). As the protein content increased, with doubling time of about 1.5 days, the total enzyme activity showed a more marked difference between day and night-time.

Table 1 Effect of conditioned medium on *N*-acetyltransferase activity in cell cultures

Medium added	<i>N</i> -acetyltransferase pmol per mg protein per 10 min
Medium unchanged	313 $\pm$ 17
Daytime conditioned medium	344 $\pm$ 41
Night conditioned medium	361 $\pm$ 22

Conditioned medium was taken from the 3-day cell culture during daytime (3 p.m.) or at night (3 a.m.) in darkness, and was added to other 3-day cell cultures next day at 11 a.m. The incubations were continued until 5 p.m. and *N*-acetyltransferase activity was measured. The results are means $\pm$ s.e.m. of five samples.

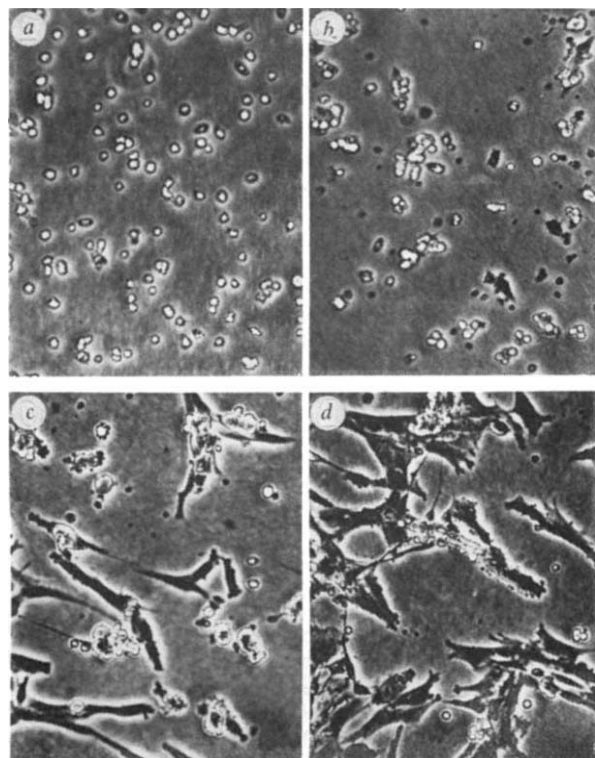


Fig. 1 Phase contrast micrographs of dispersed pineal cells shortly after inoculation (a), after 24 h (b), 48 h (c), and 60 h (d) of culture. Scale bar, 100 μ m. Eighty to ninety chickens were killed between 2 and 3 p.m. by decapitation. The pineal glands were freed of connective tissues and severed into small pieces by pincettes under a stereo microscope. The pineal tissues were incubated in a 40-ml tube containing 25 ml of incubation mixture at 37 °C with constant shaking. The incubation mixture consisted of 25 mM HEPES-NaOH (pH 7.2), 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 10 mM glucose, 0.36 mM CaCl_2 , streptomycin (100 $\mu\text{g ml}^{-1}$), penicillin (100 U ml^{-1}), 1% bovine serum albumin, and 0.4% collagenase (type I) from *Clostridium histolyticum* (Sigma). During the incubation of 15–20 min, the pineal pieces were triturated by splashing the suspension against the tube wall through a thin Pasteur pipette. Undispersed fragments of the tissues were sedimented by standing the suspension for 10–15 min. The cell suspension was transferred into another tube and the cells were collected by centrifugation at 1,000g for 5 min. The cells were washed with 30 ml of culture medium 3 times followed by centrifugation. Suspension (4 ml) containing $1.4\text{--}1.6 \times 10^6$ cells was inoculated in a Falcon plastic culture dish (5 cm diameter). Culture medium was the same as that used for organ cultures¹¹. Monolayer cultures were carried out at 40 °C in a humidified atmosphere of 10% CO_2 and 90% air in a transparent incubator box placed in a CO_2 incubator. Inside of the incubator was kept dark. When indicated, a fluorescent lamp (30 W) and electric bulb (100 W) provided light through a glass door of the incubator. Intensity of the illumination was 800–1,100 lux at dish level. The culture medium was changed every 3 days.

After 3 days of culture, fibroblasts covered most of the surface of dishes, and the specific enzyme activity per mg protein decreased, although the total enzyme activity per dish was maintained for another several days (data not shown).

To examine whether the rhythmic change of *N*-acetyltransferase activity was due to a synchronised cell cycle, cytosine-1- β -D-arabinofuranoside (1×10^{-5} M) was added to the medium on day 3 of culture to inhibit cell division (Fig. 3b). The activity of *N*-acetyltransferase still showed a 10-fold increase at night compared to the daytime value. In another experiment, cells were cultured for 14 days (17 days after the start of culture) in the presence of cytosine-1- β -D-arabinofuranoside. *N*-Acetyltransferase activity continued to oscillate rhythmically (on day 17 of culture, daytime values at 4 p.m.,

304 ± 15 ; night values at 4 a.m., $2,733 \pm 160$ pmol per mg protein per 10 min).

Dispersed cells were cultured under the LD cycle for 2 days and on the third day the lights were turned off at 1 p.m., 6 hours before the onset of usual darkness (Fig. 3c). *N*-Acetyltransferase activity did not increase during the subjective daytime, but increased at 10 p.m., reaching a maximum at midnight. In the experiment shown in Fig. 3d, cells were cultured in continuous darkness from the second day for two successive days. There were rhythmic changes of two cycles with a higher enzyme activity during the subjective night-time compared to that of the subjective daytime. However, the day-night difference was damped out on the third day of culture: the daytime levels being higher and the night-time values being lower than those of the cells cultured in the LD cycle (Fig. 3a).

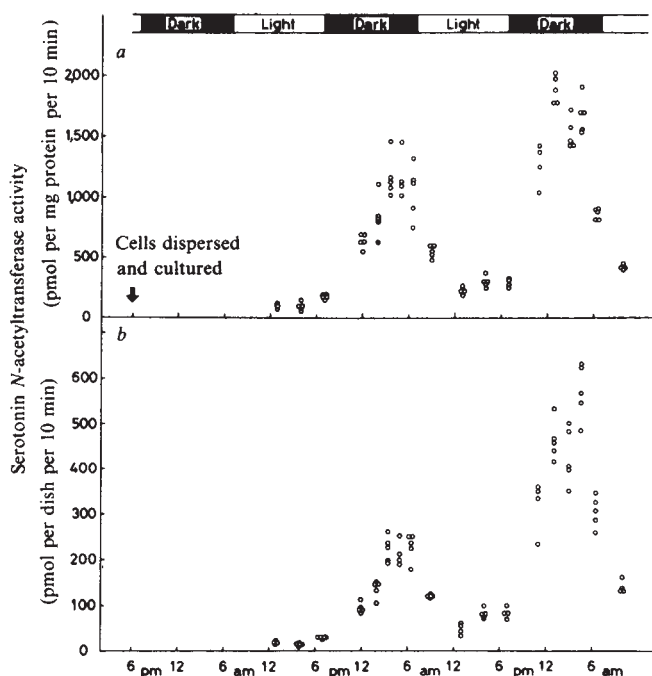


Fig. 2 *N*-Acetyltransferase activity in the dispersed cells cultured in a light-dark cycle. Cultured cells were scraped with a rubber policeman, transferred in a centrifuge tube together with the culture medium, and collected by centrifugation at 1000g for 10 min. The pellet was homogenised in 65 μ l of 0.05 M potassium phosphate buffer (pH 6.5) by use of a small glass homogeniser. The homogenate (50 μ l) was used for the assay of *N*-acetyltransferase activity¹¹ and the rest (15 μ l) for the determination of protein by the method of Lowry *et al.*¹⁷. a, Specific enzyme activity (pmol per mg protein per 10 min); b, total enzyme activity (pmol per dish per 10 min). Each point represents the enzyme activity of individual dish.

When cells were exposed to continuous illumination in the third night, the increase of enzyme activity was suppressed to half or less (Fig. 3e). However, there was threefold elevation of *N*-acetyltransferase activity during the subjective night-time compared to the daytime level. These observations indicate that the rhythmic change of *N*-acetyltransferase activity in the dispersed cells is not a reaction to the environmental lighting, but is generated by an endogenous oscillator in the cells. Cells were cultured for 3 days in the LD cycle inverted to the lighting schedule in which the chickens had been raised. The rhythmic change of *N*-acetyltransferase activity was entrained to the reversed lighting schedule in which the cells were cultured (Fig. 3f).

To determine whether one type of pineal cell released a transmitter or a hormone which stimulated the synthesis of

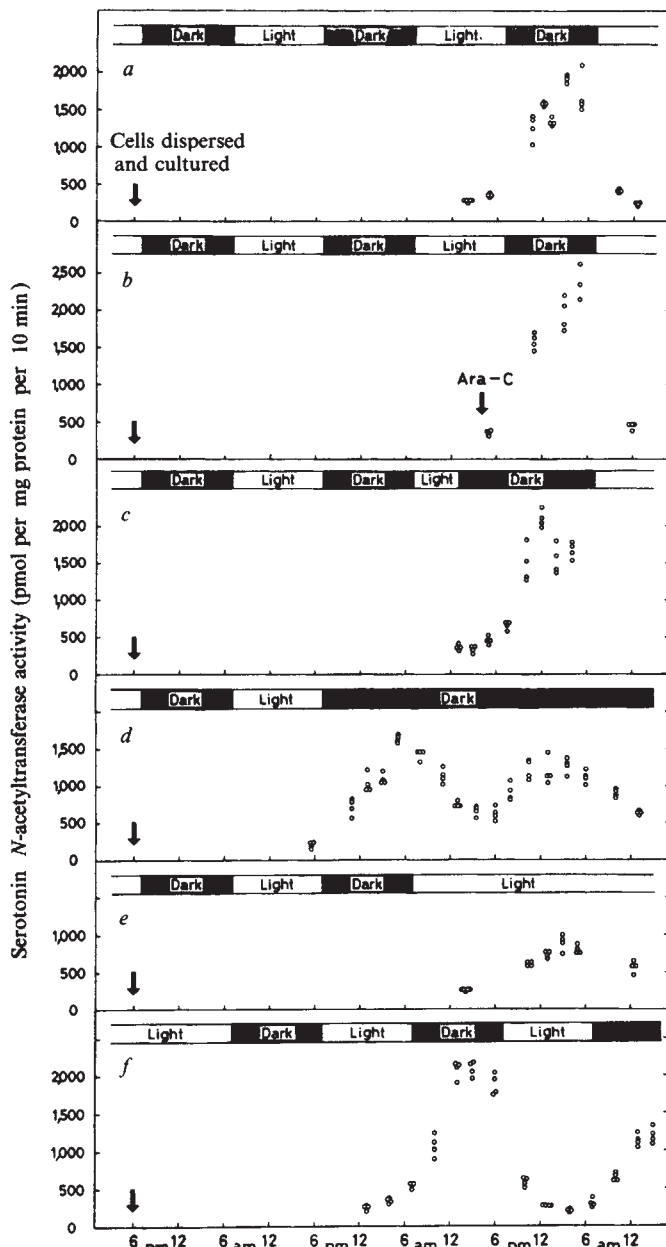


Fig. 3 *N*-Acetyltransferase activity in the pineal cells cultured under various lighting schedules. Each point represents the specific enzyme activity of an individual dish. 'Ara-C' indicates the addition of cytosine-1- β -D-arabinofuranoside (1×10^{-5} M) to the medium.

N-acetyltransferase in another type of pineal cell, conditioned medium was taken from the 3-day cell culture during daytime and at night in darkness, and was added to another cell culture at 11 a.m. when *N*-acetyltransferase activity was low. There was no significant elevation of *N*-acetyltransferase activity in both conditioned mediums (Table 1).

The present observations with the dispersed cell culture are remarkably comparable to those reported using organ culture of chicken pineal gland¹¹: (1) In a LD cycle, *N*-acetyltransferase activity continued to oscillate in phase with the lighting schedule for long periods—at least 3 days in the organ culture¹¹ and 17 days in the dispersed cell culture. However, the amplitude of the rhythm was gradually decreased in the organ culture, presumably due to a necrosis of the gland, while in the dispersed cells the same magnitude of *N*-acetyltransferase activity was maintained during the period examined. (2) When cultured in

continuous darkness, the rhythmic changes of *N*-acetyltransferase activity were damped out in both the organ culture and the cell culture. This could be due to desynchronization of the rhythms in individual cells. (3) Even when lights were turned off during the daytime, *N*-acetyltransferase activity did not increase during the subjective daytime in both organ culture and the cell culture, indicating the endogenous nature of the rhythmicity. (4) Continuous illumination of the glands or the cells suppressed the night-time increase of *N*-acetyltransferase activity, although there were still significant elevations of enzyme activity during the subjective night-time, even under illumination. (5) The rhythmic change of *N*-acetyltransferase activity was entrained to the reversed LD cycle in the dispersed cells, although this was not examined with the organ culture. (6) The night-time level of *N*-acetyltransferase activity in the organ culture was about 3,500 and 1,250 pmol per mg protein per 10 min (700 and 250 pmol per mg pineal per 10 min) in the first and second nights of culture, respectively¹¹. The night-time level of enzyme activity in the dispersed cells reported here is 1,500–2,700 pmol per mg protein per 10 min, which is the same as that of organ culture and is close to the *in vivo* rhythm¹¹. The daytime activity in the dispersed cells was also comparable to those of intact pineal gland. If the *N*-acetyltransferase rhythm in the dispersed cells is due to the formation of cellular interaction in small clusters, the maximum enzyme activity should be much lower than those of intact glands, because only a portion of the cells form clusters (Fig. 1). To further rule out the possibility that the reaggregated cells re-establish the specific cell-cell interaction to induce the circadian rhythm of *N*-acetyltransferase activity, dispersed cells were inoculated at different cell populations from 5×10^5 to 4×10^6 cells per dish to give different degrees of reaggregation. Activity of *N*-acetyltransferase showed a similar night-time increase at various cell populations (data not shown).

It has been well demonstrated that, although dispersed embryonic cells reconstruct tissue-like structure in suspension or rotation cultures, the cells rapidly lose the histoformative tendency in monolayer cultures^{14–16}. Cells from embryos at a late stage of gestation or from postnatal animals do not show tissue formation in cultures. Thus it is unlikely that the dispersed pineal cells studied here re-established the tissue-like cellular interaction, which in turn generated the *N*-acetyltransferase rhythm. The present observations that the dispersed cells behave in the exactly same manner as the intact pineal glands are very indicative, though not conclusive, that the photoreceptor, the circadian oscillator and the melatonin-synthesising machinery reside in the same cell of chicken pineal gland. This system will offer an ideal model for a long-term study of the circadian oscillator of vertebrates in culture.

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Induction of vitellogenin and growth of implanted oocytes in male cockroaches

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Vitellogenins are yolk protein precursors that are synthesised in the liver of lower vertebrates in response to ovarian hormones^{1,2}, and in the fat body tissue of insects, under the influence, in most species, of juvenile hormone (JH) from the corpora allata (CA)^{3,4}. Vitellogenins are normally restricted to females, although in male amphibians¹ and roosters² their synthesis can be induced artificially by the injection of oestrogens. Thus female specificity is maintained by hormonal differences between adult males and females. In insects, on the other hand, because the CA of adults of both sexes are active^{4,5}, it appeared that male fat body could not normally respond to JH by synthesising vitellogenin. However, precise JH synthetic rates of male CA are only known in *Schistocerca gregaria*⁶ and *Diploptera punctata*⁷, in which species they are low compared to the rates in the female glands. The absence of vitellogenin in adult males could thus be due to inadequate JH titres. We report here that synthesis of vitellogenin can indeed be induced in males of *Diploptera* by implantation of female CA or application of a JH analogue, ZR512 (Zoecon), and that implanted oocytes take up the vitellogenin.

In preliminary experiments we found that removal of the CA of newly-emerged (day 0) females ($n=5$) prevented the appearance of vitellogenin on day 6, as determined by rocket immunoelectrophoresis. Also, normal adult males from 6 to 21 days old ($n=39$) contained no detectable vitellogenin (titres approaching 0.1% that of normal day 6 females, or 10 ng, are detectable by this technique). A pair of CA from day 0 females was then transplanted to day 0 males with or without a single ovary, also taken from day 0 females. Haemolymph of the males was subsequently checked for vitellogenin on days 3–10 by rocket immunoelectrophoresis. We also measured vitellin in the implanted oocytes (after uptake into oocytes, vitellogenin is referred to as vitellin). Vitellogenin was detected in the haemolymph of the CA recipients from day 3 or 4 onward, and reached a peak concentration on day 8 (Fig. 1a). In those males that received only CA implants, vitellogenin titres were about twice those of males that received both CA and an ovary. This suggested that the ovary may have removed some of the protein from the haemolymph. Indeed, Fig. 1b shows that oocytes contained vitellin, which reached a maximum level by day 9, when approximately 9 μg per ovary was measured. The oocytes themselves grew, with visible yolk granule formation, and had laid down chorion by day 8. However, they were slightly smaller and contained less vitellin than either oocytes of sham-operated females or those implanted into ovariectomised females (Table 1).

When 150 μg of ZR512 was topically applied in 5 μl acetone to day 0 males implanted with an ovary, vitellin levels were much higher by day 8, approaching those of a normal female ovary (Fig. 2b). This is consistent with previous findings from this laboratory (unpublished) that ZR512 is a more potent promoter

of oocyte growth in allatectomised females than the topically applied naturally occurring JH of *Diploptera* (identified as $\text{C}_{16}\text{JH} \equiv \text{JH III}$ (ref. 8)). On the other hand, haemolymph titres of vitellogenin in ZR512-treated males were comparable to those of males implanted with CA (Fig. 2a), suggesting that the limiting step in appearance of vitellin is the synthesis of the yolk protein precursor by the fat body and not its uptake by the oocytes. When acetone alone was applied, no vitellogenin or vitellin was detected and implanted oocytes did not grow.

This is the first conclusive evidence outside Diptera on the induction of vitellogenin in adult male insects. Limited growth of oocytes implanted into males of the cockroach, *Periplaneta americana*, has been reported^{9,10} although no vitellogenin

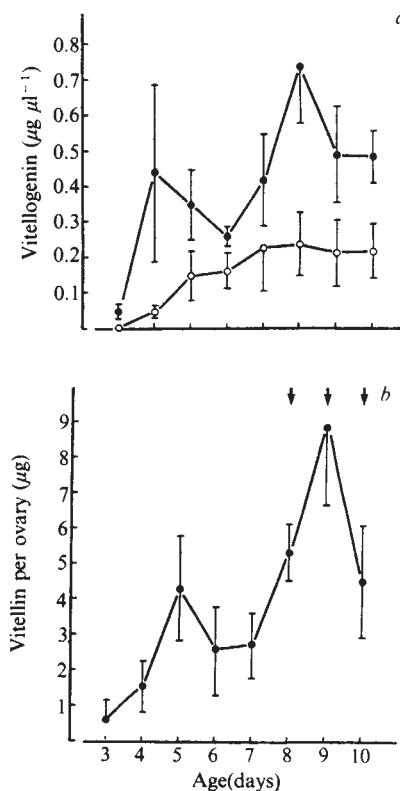


Fig. 1 a, Haemolymph vitellogenin concentration in adult males implanted at day 0 with CA of day 0 females, with (○) and without (●) one day 0 ovary (Ov). b, Total vitellin in implanted oocytes. The arrows indicate age at which 100% of the oocytes were chorionated. Each point represents the mean of 4–6 determinations (n) except day 10 when $n=11$. Vertical bars represent one standard error of the mean. Corpora allata were dissected free of adhering fat body tissue and implanted into the neck region of the male recipients. Ovaries were implanted into the abdominal cavity by injection in a small volume of Yeager's Ringer through the intersegmental membrane using a glass needle. Vitellogenin and vitellin titres were determined by rocket immunoelectrophoresis²⁶. Antiserum against egg homogenates was raised in rabbits and was rendered specific to vitellin by absorption with male haemolymph. Specificity of the absorbed antiserum was demonstrated by a single immunoprecipitation reaction in agarose against both vitellin of egg extracts and vitellogenin of female haemolymph after separation in polyacrylamide gels. Vitellogenin and vitellin were found to be immunologically identical by Ouchterlony double diffusion. Absorbed antiserum was mixed with melted agarose (1% in Tris-buffered 0.025 M Tricine, pH 8.6; Bio-Rad) at 50 °C for a final concentration of 0.25–1.00% (v/v), depending on titres of antigen to be tested. Slabs (1.5 mm thick) were then cast, wells cut, and 10- μl samples applied. Electrophoresis was carried out for 12 h at 150 V in a water-cooled chamber (Miles). Immunoprecipitation rockets were quantitated using egg extracts of known protein concentrations as determined by a micro-biuret procedure²⁷. Vitellin makes up ~90% of the soluble egg extract (E.C.M., S.S.T. and B.S., in preparation).

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synthesis was detected¹⁰. Similar results were obtained with *Eublaberus posticus*¹¹. In another cockroach, *Nauphoeta cinerea*, both ovaries and female CA were implanted into males but no oocyte growth resulted¹². After transplantation of oocytes to males of the Lepidoptera, *Bombyx mori*^{13,14} and *Pieris brassicae*¹⁵, oocyte development took place, but again, no vitellogenin was detected. Conversely, in *Hyalophora cecropia*, vitellin was detected in implanted oocytes¹⁶. This finding led to the discovery of minute quantities of the yolk protein precursor in normal adult males (0.1% that of females)¹⁶. Similar quantities were detected in *Oncopeltus fasciatus*¹⁷.

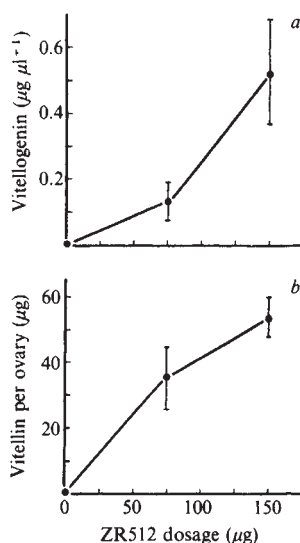


Fig. 2 *a*, Haemolymph vitellogenin concentration in 8-day-old adult males implanted with one ovary at day 0 and topically treated with ZR512 in 5 µl acetone at doses of 75 µg ($n = 8$) and 150 µg ($n = 9$), and with acetone alone ($n = 8$). Procedure as for Fig. 1. *b*, Total vitellin in implanted oocytes on day 8. Procedure as for Fig. 1.

In the Dipterans, *Sarcophaga bullata* and *Drosophila melanogaster*, vitellogenin synthesis in adult males can be induced^{18,19}. In *Sarcophaga*, injection of ecdysone reportedly led to the appearance of vitellogenin, whereas JH had no effect. However, implanted oocytes did not mature¹⁸. In contrast, males of *Drosophila* were induced to synthesise vitellogenin by implantation of ovaries and oocytes apparently matured completely²⁰⁻²². Synthesis of vitellogenin in *Drosophila* is reported to require a factor from the ovaries¹⁹ and the ovaries themselves may be a source of vitellogenin²³. The appearance of vitellogenin in *Diploptera* reported here occurred even when ovaries were not present. Clearly, in Diptera, yolk protein synthesis is controlled differently, both with regard to the role of JH and that of the ovaries.

It is evident that the adult male fat body of *Diploptera* has the capacity to synthesise vitellogenin when stimulated by sufficient titres of JH. It has also been reported that normal adult males of *Rhodnius prolixus* produce yolk protein precursor^{24,25}, often in

amounts similar to females (Engelmann and Mundall, unpublished). These findings, together with the identification of small amounts of vitellogenin in *Hyalophora*¹⁶ and *Oncopeltus*¹⁷ adult males, suggest that the capacity of males to synthesise vitellogenin is not anomalous among insects.

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Is multiple sulphatase deficiency due to defective regulation of sulphohydrolase expression?

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Multiple sulphatase deficiency disease is an unusual autosomal recessive disorder characterised biochemically by a deficiency of several sulphohydrolase activities^{1,2}. The laboratory diagnosis of this combined neurological connective tissue disorder is made on the basis of decreased activities of the lysosomal enzymes, arylsulphatase A and arylsulphatase B and the microsomal enzyme, arylsulphatase C. The primary defect in this multi-enzyme deficiency has not been identified. Using immunological techniques to characterise further the residual activities of arylsulphatases A and B in the multiple sulphatase deficiency disease, we have examined the levels of cross-reacting material (CRM) to arylsulphatases A and B in cultured skin fibroblasts from controls and patients with multiple sulphatase deficiency, metachromatic leukodystrophy (deficiency of only arylsulphatase A activity) and Maroteaux-Lamy syndrome (deficiency of only arylsulphatase B activity). We report here results indicating that arylsulphatases A and B in multiple sulphatase deficiency are reduced in their levels of CRM while retaining a normal activity/CRM ratio. Because the two enzymes are apparently structurally unrelated, these data are consistent with the possibility that their combined deficiencies in this disorder may result from a defect in the coordinated expression of sulphohydrolases.

Table 1 Total vitellin on day 7 in ovaries (Ov) re-implanted into 0 day ovariectomised females (OvX) and those of sham-operated controls*

Treatment	Vitellin (µg per ovary)	n
OvX + Ov	84.4 ± 16.2	5
sham-operated	83.2 ± 11.9	5

Values are mean ± s.e.m.; n, numbers of animals.

* Haemolymph vitellogenin titres were not determined.

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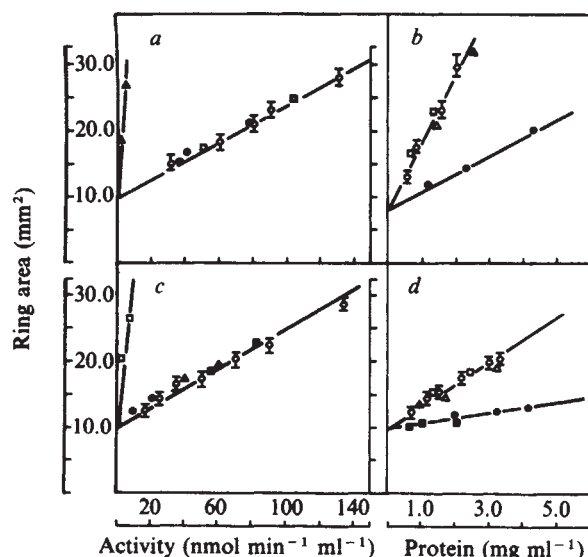
Skin fibroblasts from patients with multiple sulphatase deficiency ($n=3$), metachromatic leukodystrophy ($n=2$) and Maroteaux-Lamy syndrome ($n=2$), as well as controls ($n=5$), were grown as previously described. Cultures were also maintained in HEPES- CO_2 as described by Fluharty *et al.*³, who reported that with growth in this medium, arylsulphatases A and B in multiple sulphatase deficiency returned to normal activity levels: we failed to confirm this observation. Cells were collected by trypsinisation at confluency and lysates containing arylsulphatases A and B were obtained by freeze-thaw and centrifugation. Enzyme assays confirmed the diagnosis of multiple sulphatase deficiency; compared with control, lysates from multiple sulphatase deficiency cell line 1 had <1% arylsulphatase A, ~5% arylsulphatase B; those from multiple sulphatase deficiency cell line 2 had ~7% arylsulphatase A, ~10% arylsulphatase B; and those from multiple sulphatase deficiency cell line 3 had ~5% arylsulphatase A, ~10% arylsulphatase B; arylsulphatase C was 40–60% of control in these lines. In some experiments, arylsulphatase A and B activities were separated by anion exchange chromatography². No cross-reactivity was observed between purified arylsulphatase A and arylsulphatase B to anti-arylsulphatase A and anti-arylsulphatase B, respectively, and therefore intact lysates were also used. Ouchterlony double diffusion demonstrated single lines of identity between normal fibroblast lysates and lysates from multiple sulphatase deficiency cells when tested with either anti-arylsulphatase A or anti-arylsulphatase B. These results validated the use of more quantitative tests to measure the relative levels of CRM to arylsulphatases A and B in the various cell lines.

Figure 1 shows the results of single radial immunodiffusion (SRID) assays⁴. Figure 1a plots the anti-arylsulphatase A as ring area against activity. Lysates from multiple sulphatase deficiency cells fell on the same line as controls and Maroteaux-Lamy syndrome cells, whereas the slope for lysates from metachromatic leukodystrophy cells was steeper, indicating considerably less enzymatic activity per CRM than controls, multiple sulphatase deficiency or Maroteaux-Lamy syndrome. Figure 1b plots the anti-arylsulphatase A assay ring area against protein in the assayed samples. The multiple sulphatase deficiency lysate fell below the line obtained for control,

metachromatic leukodystrophy and Maroteaux-Lamy syndrome lysates. Figure 1c, d illustrates the data obtained for SRID assays carried out with anti-arylsulphatase B. The data are plotted as described above for anti-arylsulphatase A, with similar results in both plots for two multiple sulphatase deficiency lysates. In this assay, Maroteaux-Lamy syndrome lysates showed considerably lower activity/CRM ratios than controls, multiple sulphatase deficiency and metachromatic leukodystrophy (Fig. 1c). Taking all the results of Fig. 1 together, the following conclusions may be made. (1) Arylsulphatase A and arylsulphatase B from multiple sulphatase deficiency cells are similar to controls with respect to enzymatic activity per unit CRM; (2) arylsulphatases A and B are reduced in their CRM levels (in approximate proportion to the decrease in the activity observed in lysate enzyme assays) and, in contrast, (3) the arylsulphatase A in metachromatic leukodystrophy lysates and the arylsulphatase B in Maroteaux-Lamy syndrome lysates are markedly reduced in the CRM-specific enzymatic activity, although CRM to each enzyme is found at normal levels in the cells.

To confirm the observation that the CRM/activity ratio of arylsulphatases A and B in multiple sulphatase deficiency cells was comparable to that observed with normal enzyme, a sensitive competitive precipitin assay was carried out (see Fig. 2 legend for assay method). In Fig. 2, the curves described as 'expected' (x) in each graph represent the anticipated values of residual supernatant activity if the multiple sulphatase deficiency activities have a normal ratio of activity to CRM. Values significantly above expected would indicate the presence of higher amounts of CRM than indicated by the level of enzymatic activity. For both arylsulphatases A and B, values at all antibody concentrations were very close to expected. Identical agreement to expected was also obtained when the level of multiple sulphatase deficiency activity was one-half the normal activity in the assay. These results confirm the findings of the SRID assay in that the enzyme which is present in multiple sulphatase deficiency cells seems to be normal in its ratio of activity to CRM and thus, by implication from this assay, is reduced in the level of arylsulphatases A- and B-specific CRM in the cell.

Fig. 1 Single radial immunodiffusion (SRID) assays of fibroblast lysates from controls and patients with multiple sulphatase deficiency, metachromatic leukodystrophy and Maroteaux-Lamy syndrome. Antibodies to purified human liver arylsulphatase A and arylsulphatase B were raised in rabbits by multi-site injection with Freund's complete adjuvant. Arylsulphatase A and B activities were assayed as previously described⁴. Arylsulphatase B was purified as previously reported¹⁰, and arylsulphatase A was purified as follows. Liver was obtained at autopsy, homogenised in 10 mM Tris-HCl, pH 7.4 (2:1 v/w), frozen and thawed three times, sonicated briefly, and filtered through gauze; the homogenate was mixed at 4 °C with DE-cellulose equilibrated with the above buffer (1 g DE-cellulose per 2 g wet weight tissue). The adsorbed fraction, free of arylsulphatase B, was eluted batchwise with 1.0 M NaCl, the pH was adjusted to 6.0 and the fraction was mixed at 4 °C and 2 g concanavalin A bound by CNBr (ref. 11) to 200 ml Sepharose. The adsorbant was then poured into a column, washed with 20 mM Tris-acetate, pH 6.0 and 0.5 M NaCl, and enzyme was eluted with 1.0 M α -CH₃-glucoside, pH 7.4, at 22 °C. Activity fractions were concentrated by negative pressure ultrafiltration, dialysed against 20 mM Tris-acetate, pH 6.0, and applied to a column of Cibacron Blue-GioGel 0.5 M (100 cm $\times$ 2 cm)¹². Peak fractions were pooled and fractionated on a DE-cellulose column, equilibrated in 10 mM Tris-HCl, pH 7.6, and eluted by a 0–500 mM NaCl linear gradient. Final purification was accomplished by fractionation on a column of BioGel P-150 (90 cm $\times$ 1.0 cm) at pH 7.4 (50 mM Tris-HCl). Purity of the enzyme was demonstrated by polyacrylamide electrophoresis¹³ and a symmetrical peak of constant specific activity on gel filtration. Antibodies to arylsulphatase A and arylsulphatase B and their respective IgG fractions prepared by precipitation of the antiserum with 40% saturated ammonium sulphate, appeared monospecific by immunoelectrophoresis against the original liver material. These assays were carried out in Petri plates of 1.5% agarose (12 ml) containing 225 $\mu\text{g ml}^{-1}$ anti-arylsulphatase A IgG or 125 $\mu\text{g ml}^{-1}$ anti-arylsulphatase B IgG and 0.02% $\text{Na}_2\text{S}_2\text{O}_4$; 7- μl samples were applied to each well and allowed to diffuse for 48 h at 37 °C. Plates were washed in phosphate-buffered saline for 72 h and, to increase the sensitivity of the assay, a second antibody, goat-anti-rabbit IgG, was applied to each well and allowed to diffuse in a similar manner. Antibody-antigen precipitin rings were measured with a Bausch and Lomb calibrated eyepiece. a, b, SRID using anti-arylsulphatase A (anti-ASA); c, d, SRID using anti-arylsulphatase B (anti-ASB). Scale for protein represents actual values for SRID with anti-arylsulphatase A and standardisation to 1.0 mg ml^{-1} for anti-arylsulphatase B (actual values $\times 3$). $\circ$, Controls ($\pm$ range, $n=4$); $\blacksquare$, multiple sulphatase deficiency, cell line 1; $\bullet$, multiple sulphatase deficiency, cell line 2; Δ , metachromatic leukodystrophy; $\square$, Maroteaux-Lamy syndrome. (The results from two mutant lines are pooled for metachromatic leukodystrophy and Maroteaux-Lamy syndrome.)



To examine the possibility that the intracellular activities of arylsulphatases A and B in multiple sulphatase deficiency are reduced because of excessive secretion into the media, control and multiple sulphatase deficiency fibroblasts (cell lines 2 and 3) were examined for levels of arylsulphatase A and B activities in serum-free media in conditions previously described⁵. No differences in arylsulphatase A or B activities were observed between control and multiple sulphatase deficiency cells (~10 nmol per min per mg cell protein). In addition, no differences were observed in the migration of arylsulphatase A and B activities from control and multiple sulphatase deficiency lysates on polyacrylamide gel electrophoresis at pH 4.1.

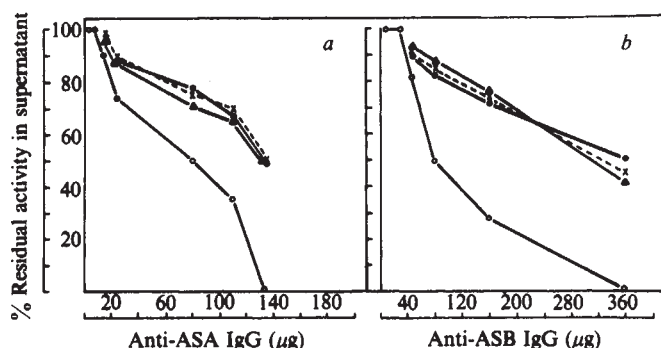


Fig. 2 Competitive precipitin assays of multiple sulphatase deficiency compared with control fibroblast lysates. A precipitin curve for normal enzyme was first established. Increasing amounts of antibody were added to a constant amount of cell lysate (that is, a constant level of activity), incubated first at 37°C for 120 min then at 4°C for 16 h. A previously formed complex (100 µg) of human serum albumin (HSA)-anti-HSA was added to each tube as a carrier protein, the mixture centrifuged at 10,000g for 20 min and the supernatants assayed for unprecipitated enzymatic activity. Equal levels of enzymatic activity from multiple sulphatase deficiency lysates were then added to tubes containing the previously defined ratio of antibody to normal cell lysate. *a*, Assay of arylsulphatase A using anti-arylsulphatase A; *b*, assay of arylsulphatase B using anti-arylsulphatase B. ○, Control; ●, multiple sulphatase deficiency, line 2; ▲, multiple sulphatase deficiency, line 3; ×, expected values (see text for discussion).

these enzymes may be accounted for by their elevated levels extracellularly⁸. Thus, these studies suggest the existence of a unique monogenic disorder in humans which is the result of a defect in a process which coordinates the expression of several enzymes.

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Inefficient lactate dehydrogenases of deep-sea fishes

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The respiratory rates of deep-sea animals are extremely low. Deep-sea fishes may consume oxygen at rates only 5-10% those characteristic of shallow-water species¹⁻⁴. These low metabolic rates are probably an adaptation to the presumed scarcity of food in deeper water⁴. The biochemical basis of this metabolic adaptation is a low level of enzyme activity in the skeletal muscle (but not the heart or brain), mainly because of the low enzyme concentrations in that tissue⁵. We report here, however, that a second source of reduced enzyme activity contributes to the low metabolic rate. For muscle-type (M₄) lactate dehydrogenases (LDH, EC 1.1.1.27, NAD⁺:lactate oxidoreductase), the enzymes of deep-sea fishes have significantly higher activation free energy (ΔG^*) and enthalpy (ΔH^*) characteristics than the homologous enzymes of cold-adapted, shallow-water fishes. Because of these higher energy barriers to catalysis, pyruvate is reduced to lactate at approximately 60% of the rate observed with LDHs of shallow-water fishes. Thus, in terms of rate of function per enzyme molecule, deep-sea fishes would be at a disadvantage in shallow waters because of their relatively poor capacity for muscle glycolysis. Such enzymatic factors may help determine the upper distributions of deep-sea species, much as the relatively large pressure insensitivities of LDHs of these deep-sea fishes^{6,7} may enable them to tolerate high and variable pressures. We suggest that the low catalytic efficiencies of high-pressure-adapted LDHs are concomitant with their low sensitivities to pressure.

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Hypotheses to explain the defect in MSD include (1) a mutation in a subunit or 'factor' common to the sulphohydrolases; (2) the presence of an inhibitor of the affected sulphohydrolases derived from a defect in another metabolic pathway; (3) a defect in a cellular process which coordinately regulates (pre- or post-translationally) the expression of sulphohydrolases. The demonstration that catalytically normal arylsulphatases A and B are present in reduced concentrations in multiple sulphatase deficiency is most consistent with the third hypothesis; these findings are also supported by recent immunological studies by Fluharty *et al.*³, who observed reduced levels of CRM to arylsulphatase A in multiple sulphatase deficiency. It cannot be determined, however, whether arylsulphatases A and B are decreased in their synthesis or increased in their rate of degradation; the latter may occur, for instance, through a mutation in a putative stabiliser molecule common to the enzymes, a defect which may result in a decay of the sulphatases at both the antigenic and activity levels. These findings in multiple sulphatase deficiency are in contrast to the apparent mutation in the structural genes for arylsulphatase A in metachromatic leukodystrophy and arylsulphatase B in Maroteaux-Lamy syndrome⁶. The recent localisation of arylsulphatase A to chromosome 22 and arylsulphatase B to chromosome 5, as well as the independent expression of each enzyme, provides evidence against a common structural feature between the two enzymes⁷. It is difficult to reconcile our findings with either of the first two explanations. Although mucopolipidosis II (I-cell disease) is characterised by the intracellular deficiency of several lysosomal enzymes in cultured skin fibroblasts, the majority of

Studies of homologous enzymes from animals adapted to different temperatures have shown that ΔH^* and ΔG^* are lowest for reactions of the most cold-adapted species⁸⁻¹². These differences influence reaction rates and are viewed as adaptive, as the lower energy barriers in cold-adapted animals facilitate metabolic rate compensation to temperature. Because the temperatures of the deep sea average only 2–5 °C (ref. 13), one might predict, on the basis of temperature adaptation studies, that deep-sea animals would possess highly efficient enzymes. For M_4 -lactate dehydrogenases this expectation is not realised (Fig. 1, Table 1). The Arrhenius activation energies (E_a) of the deep-sea species are homogeneous (analysis of variance (ANOVA), $F_{[3,19]} = 0.99$, $0.5 < P < 0.25$), and the E_a of the shallow-living, cold-adapted species are homogeneous ($F_{[1,19]} = 2.24$, $0.25 < P < 0.10$). However, the E_a values of the deep and shallow species groups differ ($F_{[1,19]} = 9.09$, $P < 0.01$). The M_4 -LDHs of deep-sea teleosts belonging to four families are consistently found to have higher activation energy characteristics and lower rates of activity per enzyme molecule (Table 1; see legend for computation procedures) than the M_4 -LDHs of shallow-living, cold-adapted species. When compared at 5 °C and 1 atm pressure, M_4 -LDHs of deep-sea fishes reduce pyruvate to lactate at only about 60% of the rate of LDHs of cold-adapted, shallow-living fishes (Table 1). Thus deep-sea fishes seem to have a low capacity for anaerobic glycolysis, in comparison with shallow species, in conditions of low hydrostatic pressure and low temperature. However, at deep-sea pressures, the reduced pressure sensitivities of M_4 -LDHs of deep-sea fishes may compensate for the low efficiencies of these enzymes^{6,7}; at these pressures M_4 -LDHs of shallow-living fishes show significant velocity inhibition and perturbation of K_m values^{6,7}.

The importance of hydrostatic pressure as a selective factor in enzyme evolution is shown particularly clearly by the M_4 -LDHs of the two *Sebastes* congeners, *S. alascanus* (shallow) and *S. altivelis* (deep). These two species are very similar morphologically and ecologically^{6,14}, but they experience different pressure regimes at their depths of maximal abundance (Table 1). The difference in habitat pressure (of the order of 50–100 atm, corresponding to differences of 500–1,000 m in depths of distribution) seems to be large enough to involve selection for altered activation parameters, as well as for different pressure sensitivities in K_m values for pyruvate and NADH^{6,7}. Despite these significant differences in kinetic properties, M_4 -LDHs of the two species could not be distinguished by any of the electrophoretic procedures we reported previously⁶. Evidently, such electrophoretic identity may belie important functional differences between homologous enzymes.

There could be several possible explanations for reduced catalytic efficiencies of enzymes of deep-sea animals. First, if reduced metabolic rates in such a food-poor environment are selectively advantageous, then selection might favour enzymes having relatively low rates of activity¹⁵. However, we do not support this interpretation, as reduced metabolic activity could be achieved more economically by maintaining lower concentrations of more efficient enzymes. Comparisons of muscle enzyme activities (including LDH) in deep- and shallow-living fishes have shown that enzymatic protein concentrations decrease rapidly as a function of the species' minimal depths of occurrence⁵. The approximately 60% reduction of LDH activity due to inefficiency of this enzyme in deep-sea fishes could well be achieved by enzyme dilution in the cells, as an approximately 25- to 50-fold reduction in LDH activity has already been

Table 1 Activation energy parameters and relative absolute velocities of M_4 -lactate dehydrogenase reactions of differently temperature- and pressure-adapted fishes and a mammal

Species* (Depth range/body temperatures)	E_a † (cal mol ⁻¹)	ΔH^* (cal mol ⁻¹)	$\Delta S^\ddagger$ (cal mol ⁻¹ K ⁻¹)	ΔG^* ‡ (cal mol ⁻¹)	Relative velocity‡
<i>Pagotheria borchgrevinki</i> (surface/–2 °C)	11,020 (197)	10,467	–12.7	14,000	1.00
<i>Sebastes alascanus</i> (180–440 m/4–12 °C)	11,068 (196)	10,515	–12.6	14,009	0.98
<i>Sebastes altivelis</i> (550–1,300 m/4–12 °C)	12,538 (216)	11,985	–8.1	14,249	0.64
<i>Coryphaenoides acrolepis</i> (1,460–1,840 m/2–10 °C)	12,366 (220)	11,813	–8.7	14,222	0.67
<i>Halosaurus macrochir</i> (~2,300 m/2–5 °C)	12,396 (259)	11,843	–8.6	14,227	0.66
<i>Antimora rostrata</i> (1,300–2,500 m/2–5 °C)	13,110 (223)	12,557	–6.4	14,343	0.54
<i>Thunnus thynnus</i> (bluefin tuna) (surface <300 m/15–30 °C)	11,937 (153)	11,384	–10.0	14,152	0.76
Rabbit (37 °C)	13,100	12,550	–6.4	14,342	0.54

* The depth ranges given for the species are the depths of maximal abundances (see refs 4, 6 and 7 for additional data). For the analysis of variance tests, *P. borchgrevinki* and *S. alascanus* were the shallow-living species, and *S. altivelis*, *H. macrochir*, *A. rostrata* and *C. acrolepis* were the deep-living species.

† The Arrhenius activation energy (E_a) values were computed from the slopes, determined by linear regression, of plots such as those shown in Fig. 1. Standard errors for the E_a values are given in parentheses beneath the E_a values. The activation enthalpy (ΔH^*) values are for 5 °C ($\Delta H^* = E_a - RT$).

‡ Estimates of $\Delta S^\ddagger$ and ΔG^* were computed using the following rationale. For all sets of homologous enzymes that have been studied to determine activation parameters, a highly regular covariation between ΔH^* and $\Delta S^\ddagger$ has been found⁸⁻¹². For LDHs a plot of ΔH^* against $\Delta S^\ddagger$ gives a slope of approximately 330 K (refs 9, 11). This slope is called the 'compensation temperature' (ref. 20) to emphasise that simultaneous increases in ΔH^* and $\Delta S^\ddagger$ have compensating-offsetting effects on ΔG^* ($\Delta G^* = \Delta H^* - T\Delta S^\ddagger$). We assume that all of the M_4 -LDHs used in this study fit the compensation relationship noted for other vertebrate M_4 -LDHs. On this assumption we have estimated $\Delta S^\ddagger$ values, using experimentally determined differences in ΔH^* , and the experimentally determined $\Delta S^\ddagger$ from ref. 10 for flounder M_4 -LDH (the ΔH^* reported for the latter enzyme is 10,500 cal mol⁻¹). For each 330 cal mol⁻¹ increment in ΔH^* , the compensation relationship predicts a 1 entropy unit (cal mol⁻¹ K⁻¹) increase in $\Delta S^\ddagger$. Thus, using the flounder M_4 -LDH reaction as a baseline, we have computed the other $\Delta S^\ddagger$ values and, using these, the ΔG^* values. The relative velocity values are the reaction rates computed from the differences in ΔG^* among the M_4 -LDHs, using the reaction rate of the *Pagotheria* M_4 -LDH reaction (the reaction having the lowest ΔH^* and ΔG^* values) as the reference (value = 1.0). The calculated relative rates agree extremely well with experimentally determined rates for the M_4 -LDH reactions of *Sebastes alascanus* and *S. altivelis*. The specific activities of these two enzymes, in μ mol NADH oxidised to NAD⁺ per mol LDH per min, are 4.66×10^{10} and 2.82×10^{10} , respectively at 5 °C and 1 atm pressure. Protein concentrations were determined by the technique of Sedmak and Grossberg²², using bovine serum albumin as a standard. Our decision to estimate ΔG^* values using the compensation relationship rather than through direct experimental measurement of velocities and protein concentrations is based on the fact that small amounts of enzymatically inactive protein can greatly distort specific activity values, whereas a high degree of precision is possible in the measurement of ΔH^* values. The regression coefficients for all Arrhenius plots were greater than 0.996. Our value for the ΔH^* of the *Antimora rostrata* M_4 -LDH reaction is in excellent agreement with the value reported by Baldwin *et al.*¹⁵; they obtained values for E_a and ΔH^* of 13,200 and 12,648 cal mol⁻¹ (at 5 °C), respectively. Their experimentally determined substrate turnover number for the *A. rostrata* enzyme (2.48×10^{10} μ mol NADH oxidised to NAD⁺ per mol LDH per min) is also in excellent agreement with the relative rate calculated from the activation parameters. The bluefin tuna data are from ref. 23. The rabbit data (obtained using a phosphate buffer system) are from ref. 9.

achieved through maintenance of low enzyme concentrations⁵. The alternative explanation we propose to account for the high activation parameters of enzymes from high-pressure-adapted organisms is based on an analogy between high temperature and high pressure effects on enzymes. For enzymes of high body temperature species like mammals, a concomitant of low catalytic efficiency (Table 1) is a high degree of structural stability; for example, a high resistance to thermal denaturation^{8,11,12}. This relationship has been found for LDHs, and has been interpreted in terms of additional weak bonds, for example, hydrogen bonds, stabilising the mammalian enzyme-cofactor-substrate ternary complex relative to the complex formed with a fish LDH¹¹. Elevated hydrostatic pressures may also favour selection for more structurally stable enzymes which, like the enzymes of high body temperature species, also have reduced catalytic efficiencies. In fact, the M₄-LDHs of the four deep-sea fishes have efficiencies similar to that of a mammalian M₄-LDH (Table 1).

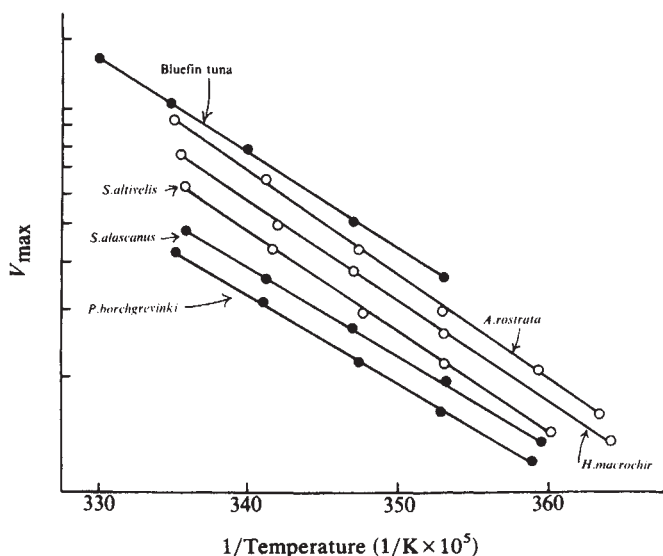


Fig. 1 Arrhenius plots (log $V_{\max}$ against reciprocal of absolute temperature (K)) for M₄-lactate dehydrogenase reactions of several deep-sea and shallow-living teleost fishes. The $V_{\max}$ values were determined using M₄-isozymes of LDH purified by oxamate affinity chromatography. Purification details are given in ref. 7. LDH activity was assayed by following the decrease in absorbance at 340 nm due to oxidation of NADH in a thermostatted spectrophotometer. Cuvette temperatures were held within $\pm 0.2^\circ\text{C}$ of the stated temperatures. The assay medium contained 80 mM imidazole/HCl buffer, adjusted to pH 6.98 at 20°C . At all of the other experimental temperatures, the pH of this imidazole buffer system follows the intracellular pH of ectothermic vertebrates (see ref. 8). The NADH concentration was 150 μM . At each temperature 6–9 pyruvate concentrations were used to determine theoretical $V_{\max}$. Data were analysed using the weighted linear regression technique of Wilkinson¹⁹. Note that the plots are positioned on the axes to show differences in slope, so the $V_{\max}$ values do not reflect the actual differences in substrate turnover number (see Table 1) of the LDHs of the shallow-living (●) and deep-living (○) fishes.

The differences in kinetic properties between M₄-LDHs of shallow- and deep-living species suggest that enzymatic adaptations may be important in establishing bathymetric distribution patterns (although biological interactions must also have an important role). Pressure-sensitive K_m values of LDHs of shallow-living fishes may make these enzymes unsuitable for function at high and/or variable pressures, and thus might help restrict the depth distributions of these species^{6,7,21}. On the other hand, even though the M₄-LDHs of deep-living fishes are very insensitive to pressure and would seem on this basis to be able to function at both shallow and great depths, the catalytic inefficiencies of these enzymes may place them at a significant disadvantage at low pressures. To possess the same glycolytic

capacity as a shallow-water fish, a deep-sea fish would have to maintain a high concentration of LDH molecules in its cells, a demand which is at odds with the low protein concentrations of the muscle fluids of these species^{5,7,16,17}.

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Post-transcriptional control in the early mouse embryo

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The earliest stages of mouse embryogenesis, from fertilisation to the two-cell stage, are characterised by an extremely low level of RNA synthesis. Indeed, during this period, RNA polymerase II activity¹ and incorporation of labelled precursors into heterogeneous RNA² are not detectable, and there is no increase in the poly(A) content of the embryo, but rather a slight decrease³. The rate of protein synthesis remains low and relatively constant throughout the one- and two-cell stages⁴. However, qualitative analysis of the protein synthetic profile on SDS gels has revealed changes which appear around the late one-cell to early two-cell stage^{4–6}. This early change in the pattern of polypeptide synthesis represents the first major qualitative molecular change found so far in development. We present evidence which suggests that the increased synthesis at the early two-cell stage of a small number of polypeptides of molecular weight 35,000 is not dependent on transcription, but rather represents control at a post-transcriptional level using mRNAs synthesised before fertilisation.

Figure 1a shows the pattern obtained when newly fertilised embryos were labelled for 3 h with ³⁵S-methionine and the radioactive polypeptides separated on a two-dimensional gel of the O'Farrell type⁷. Although there are a few notable differences between this and the pattern obtained from unfertilised eggs (Fig. 2a) the patterns are remarkably similar. The corresponding pattern from early two-cell embryos is shown in

Fig. 1b. It is clear that most of the polypeptides that are made immediately after fertilisation continue to be synthesised at the two-cell stage. In addition, among the changes which can be observed at least three polypeptides (arrowed) with molecular weights around 35,000 are made in greatly increased amounts at the later time.

If this increase was due to the *de novo* synthesis of mRNA, it should be possible to block the changes by adding a specific inhibitor of transcription. A suitable inhibitor is α -amanitin, which at levels of 10–100 $\mu\text{g ml}^{-1}$ specifically blocks mRNA synthesis, inhibits RNA polymerase II^{1,8–10} and has been shown to be effective at these doses in early mouse embryos^{11,12}. The presence of 11 $\mu\text{g ml}^{-1}$ α -amanitin in the culture medium did not prevent the first cell division, and Fig. 1c shows that it also did not block the appearance of the characteristic early two-cell

polypeptides. This result was found in four separate experiments and also when 100 $\mu\text{g ml}^{-1}$ α -amanitin was used (data not shown). However, before it can be concluded that control of the synthesis of the three polypeptides is at a post-transcriptional level, it is necessary to demonstrate that the inhibitor actually enters the cells.

As the incorporation of radioactive precursors into mRNA is undetectable over the period being studied, an indirect approach had to be adopted. It has been widely reported that α -amanitin can arrest or markedly retard cleavage in preimplantation mouse embryos^{11,13–15}. Since α -amanitin is an irreversible inhibitor¹⁶, a brief early pulse of the drug at the one-cell stage should produce effects at a later stage of development when transcription is required. As one-cell embryos from CFLP mice do not readily develop *in vitro* beyond the two-cell stage, embryos from B10 $\times$ CBA F₁ hybrids (courtesy of Dr M. H. Kaufman) were used. Table 1 shows the results from such an experiment in which newly fertilised eggs were pulsed with 11 $\mu\text{g ml}^{-1}$ α -amanitin for 6 h before they were transferred to fresh medium free of α -amanitin and allowed to develop further *in vitro*. None of the α -amanitin-treated embryos progressed beyond the two-cell stage, whereas 82% of the control embryos which reached the two-cell stage developed to the three- or four-cell stage within 56 h of fertilisation.

From this we conclude that α -amanitin does enter one-cell embryos, and that transcription by RNA polymerase II is not required for the first cell division. We also conclude from Fig. 1 that most of the mRNAs present at fertilisation are still active some 24 h later, at the early two-cell stage, and that the appearance of the new polypeptides at this stage is not due to synthesis of new mRNAs by RNA polymerase II. The changes in polypeptide synthetic profile probably therefore represent either post-transcriptional control or α -amanitin resistant transcription, as for example from a viral or mitochondrial genome.

To distinguish transcriptional from post-transcriptional control we investigated whether mRNAs coding for the 35,000-molecular weight polypeptides were present (but not translated) in unfertilised eggs. Total RNA was extracted from about 200

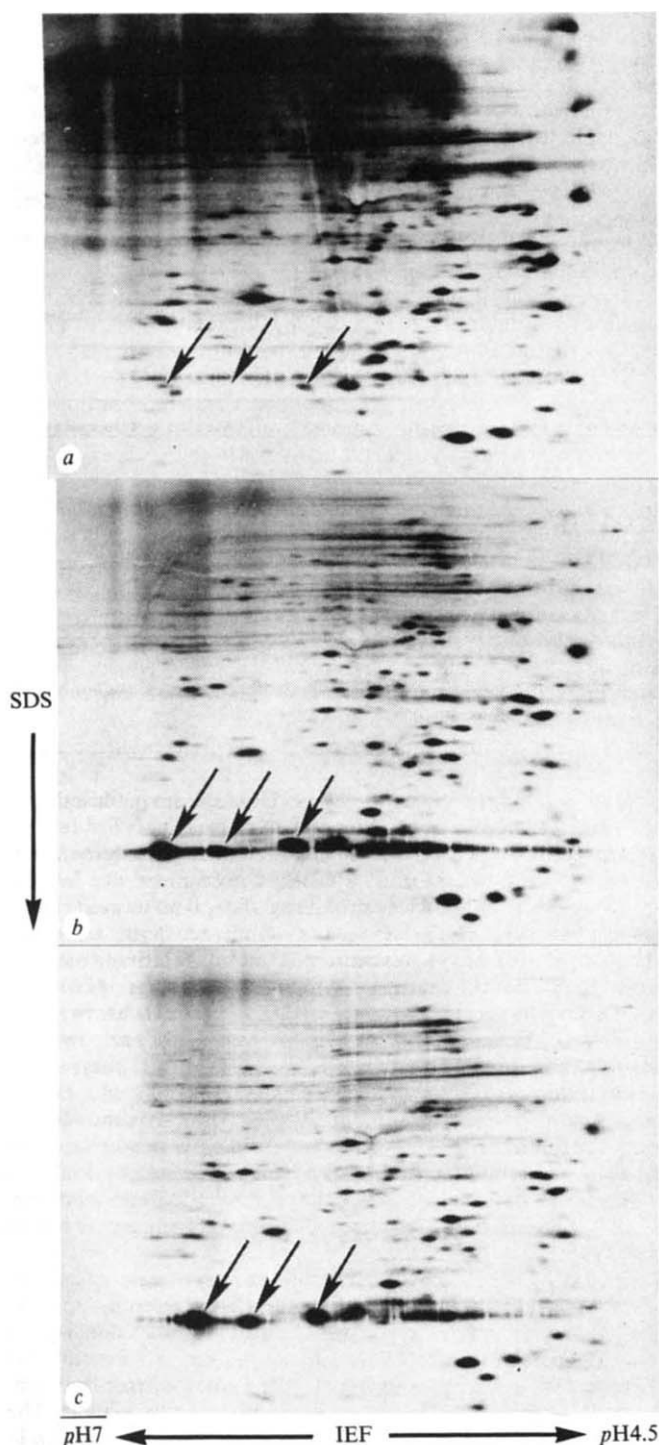


Fig. 1 Two-dimensional fluorographs of ^{35}S -methionine-labelled polypeptides from fertilised eggs (a), two-cell embryos having been cultured for 24 h *in vitro* from the one-cell stage (b), and two-cell embryos having been cultured for 24 h *in vitro* from the one-cell stage in the presence of 11 $\mu\text{g ml}^{-1}$ α -amanitin (c). Cumulus masses were recovered at 14 h after HCG from the oviducts of superovulated female CFLP mice which had been mated with CFLP males. The cumulus cells were removed by brief treatment in hyaluronidase (1 mg ml^{-1}), and were then washed through several changes of phosphate-buffered medium (PBM) containing 10% fetal calf serum²⁰. The pooled embryos were divided randomly into three groups; dead or disorganised embryos being excluded. One group was incubated for 3 h in 100 μl of medium 16 + 4 mg ml^{-1} bovine serum albumin²¹ with 10 μl of ^{35}S -methionine ($\sim 5 \text{ mCi ml}^{-1}$). At the end of this period 30 unfertilised eggs were transferred to 20 μl lysis buffer and stored at -70°C . The presence of two pronuclei and a second polar body was taken as evidence of fertilisation. The remaining two groups were incubated for 24 h in 100 μl drops of medium 16 + BSA in the presence or absence of 11 $\mu\text{g ml}^{-1}$ α -amanitin. At the end of the culture period the embryos were scored for stage of development and 10 μl of ^{35}S -methionine was added to each drop. After a further 3 h the two-cell embryos in each group were collected in 20 μl of lysis buffer. All incubations were performed at 37°C in an atmosphere of 5% CO_2 in air. Two-dimensional polyacrylamide electrophoresis in denaturing conditions was carried out over a pH range of 4.5 to 7 for the first dimension and an SDS gradient of 7–15% acrylamide for the second as described previously^{13,22}. The gels were impregnated with 2,5-diphenyloxazole²³ and exposed to preflashed²⁴ Fuji Rx X-ray film for between 2 and 6 weeks. Analysis of the film was performed as described previously¹³. The arrows indicate polypeptides weakly labelled in the fertilised egg but strongly labelled at the two-cell stage both in the presence or absence of α -amanitin.

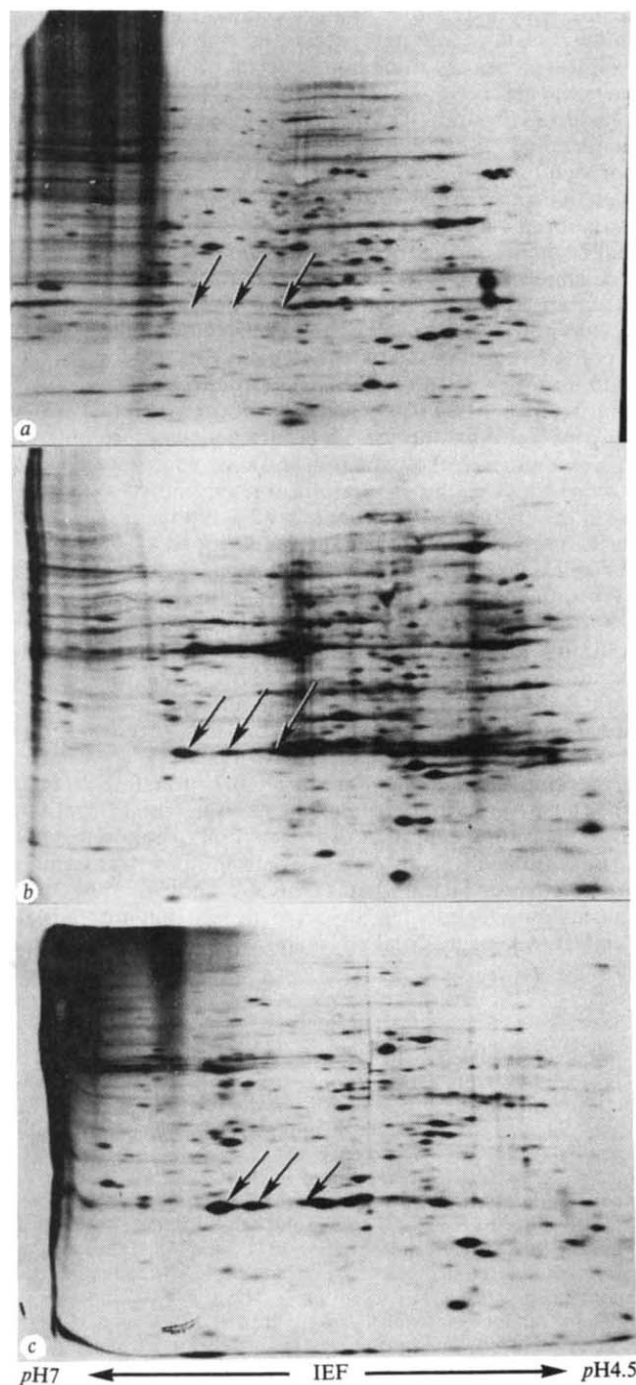


Fig. 2 Two-dimensional fluorographs of ^{35}S -methionine-labelled polypeptides from 60 intact unfertilised eggs (a), mRNA from 200 unfertilised eggs translated *in vitro* (b), and from 50 intact early two-cell embryos (c). Cumulus masses were recovered at 15 h after HCG from the oviducts of superovulated female CFLP mice. The cumulus cells were removed (as for Fig. 1), and 60 unfertilised eggs were labelled for 3 h in medium containing ^{35}S -methionine (as for Fig. 1). mRNA from 200 eggs was extracted and translated in a specially modified message-dependent reticulocyte lysate system as described previously¹⁷. Two-cell embryos were flushed from the oviducts of superovulated mice 40 h after HCG and labelled as for the unfertilised eggs. Two-dimensional electrophoresis and processing was as for Fig. 1. The arrows show polypeptides which are not strongly labelled until the two-cell stage but are translated *in vitro* from egg mRNA.

Table 1 Development of B10 $\times$ CBA 1-cell embryos* in the presence of $11\ \mu\text{g ml}^{-1}$ α -amanitin for 6 h

Stage	- α -amanitin		+ α -amanitin	
	48 h post HCG	68 h post HCG	48 h post HCG	68 h post HCG
1-cell	17	10	18	14
2-cell	23	5	25	29
3-cell	0	3	0	0
4-cell	0	20	0	0
Dead/disorganised	2	4	2	2
Total	42	42	45	45

Embryos were washed in medium free of α -amanitin at 24 h post HCG and examined at 48 and 68 h post HCG.

* 18 h after HCG administration.

unfertilised eggs and translated in the mRNA-dependent reticulocyte lysate system as described previously¹⁷. Figure 2 shows a comparison between the products obtained by *in vitro* translation and the polypeptides labelled in either intact unfertilised eggs or early two-cell embryos. The two-cell marker proteins (arrowed) were prominent amongst the cell-free products, even though they were virtually undetectable in the unfertilised egg pattern. This result was consistently found in eight experiments. It could perhaps be argued that the cell-free system might selectively translate the mRNAs for these polypeptides. However, preliminary experiments indicate that these polypeptides are no more prominent among the translation products of RNA from two-cell embryos (data not shown).

Thus, there are a few polypeptides which are prominent products of the early two-cell mouse embryos but which are only very minor products of unfertilised or newly-fertilised eggs. The appearance of these polypeptides is not blocked by the transcriptional inhibitor α -amanitin, and mRNAs coding for them are present in unfertilised eggs. Although we cannot absolutely exclude the possibility of a rapid transcriptional event occurring at fertilisation before the eggs can be transferred into the α -amanitin it seems reasonable to conclude that the increased synthesis of these polypeptides is under post-transcriptional control¹⁸. Several mechanisms for this type of control could operate¹⁹. The changes in the two-dimensional gel pattern cannot be explained merely by alteration in the charge of the polypeptides, as their appearance is also detectable on one-dimensional SDS gels. However, we cannot exclude the possibility of very rapid degradation of the polypeptides in the egg but not at the two-cell stage. Physical sequestration of the corresponding mRNAs could also explain the results, but the basis for such masking is presently unclear. Only a few polypeptides are involved, and their close proximity on two-dimensional gels suggests that they may be structurally related and under co-ordinate control. It seems that the developing embryo requires large amounts of these proteins at a time when a sudden burst of transcription is inconvenient or impossible, and therefore relies on a post-transcriptional control mechanism. In contrast, development beyond the two-cell stage seems to require some transcription of the embryonic genome (Table 1)¹³.

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Myosin isoenzyme redistribution in chronic heart overload

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Since the first observation by Spann *et al.*¹, it has become clear that in cardiac hypertrophy induced by a mechanical overloading, the velocity of shortening of the cardiac muscle (V_{max}) is reduced (see ref. 2 for review). Most authors agree that this mechanical alteration is accompanied by a decrease in the Ca^{2+} -dependent ATPase activity of myosin (see ref. 3 for review). The molecular basis of such changes was unknown because the structural modifications of the myosin molecule were ill-defined⁴⁻⁶. Nevertheless, it has recently been shown that, like skeletal muscle myosin⁷, cardiac myosin is composed of several polymorphic forms, comparable to isoenzymes^{8,9}. In the skeletal muscle, new functional requirements can induce changes in both contractile activity and type of myosin isoenzyme synthesised¹⁰⁻¹³. We now report that an increase in cardiac work produced by mechanical overloading in rats induces the preferential synthesis of a cardiac myosin isoenzyme characterised by specific immunological and electrophoretic properties and exhibiting a lower ATPase activity. This adaptive change could account for the reduced shortening speed of this hypertrophied cardiac muscle.

Cardiac hypertrophy was induced in male Wistar rats (200 g, Animalabo) either by a volume¹⁴ or pressure overload¹⁵, or by combining both procedures¹⁴, that is, abdominal aorta constriction and induction of aortic incompetence 2 weeks later (see Table 1 legend). Animals exhibiting 60 to 100% cardiac hypertrophy by any of the above procedures were selected for this study, but it should be pointed out that the most reproducible results were obtained with the two-step procedure. Enzymatic, immunological and electrophoretic tests were performed on myosins from the same hypertrophied hearts and from hearts of sham-operated animals.

Steady-state Ca^{2+} - or Mg^{2+} -dependent ATPase activities of myosins from hypertrophied hearts were both significantly depressed, whereas the K^{+} -activated ATPase remained unchanged (Table 1). This is in full agreement with earlier observations in rats^{16,17} and in other animal species^{4,18-20}. Since it has often been suggested that this diminished Ca^{2+} -dependent activity might be due to artifactual denaturation, the functional integrity of individual myosin preparations was assessed by measuring their early phosphate burst size, that is the number of

ATP moles cleaved per myosin molecule. As shown in Table 1, the myosins extracted from both hypertrophied and normal hearts cleaved 0.8 mol of ATP per active site. This value, in close agreement with previously published results for normal skeletal and cardiac muscles²³⁻²⁵, rules out the possible presence of denatured, aggregated or oxidised molecules^{23,25}. Such absence is consistent with the functional integrity of the site known as SH1 in the hypertrophied rabbit myocardium²⁰. In addition, electrophoresis on SDS-polyacrylamide gels visualised the heavy subunits as single bands (not shown) indicating that the degraded components observed by others in dogs⁶ were not present in our preparations. The decrease in ATPase specific activity was therefore not due to molecular denaturation, but more likely indicated the presence of a new myosin species with different enzymatic activities.

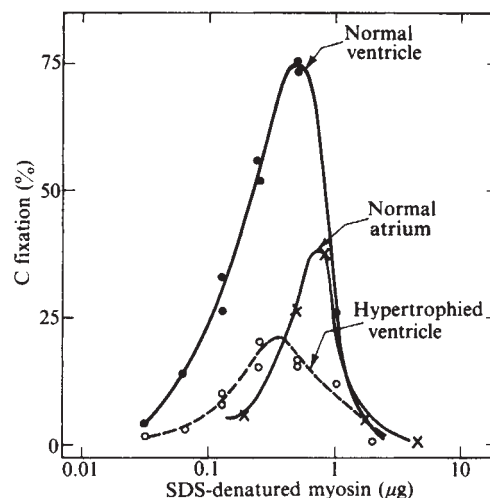


Fig. 1 Microcomplement fixations with antiserum 47-3-70 to normal rat ventricular SDS-heavy meromyosin. Antiserum dilution 1:5,250. SDS-denatured myosins were obtained from the ventricles and atria of sham-operated animals and the ventricles of a hypertrophied rat heart (100% hypertrophy preparation no. 5 in Table 1). Antibodies were raised in guinea pigs against purified rat ventricular heavy meromyosin²⁸, denatured by heating for 5 min at 90 °C in the presence of SDS (5 mg per mg protein), 2% 2-mercaptoethanol, 50 mM NaCl and 100 mM Tris-HCl, pH 7.6. Excess SDS was removed immediately before immunisation by gel filtration on a 2 × 15 cm Sephadex G-10 column²⁷. Microcomplement fixations were produced according to Levine²⁶, in a final volume of 0.7 ml. Before the assays, myosins were denatured and excess SDS was removed as described above. The mean difference between two controls was equal to 4.8%, s.e.m. = 1.7, $n = 7$. These antibodies are specific to a denatured configuration of heavy-meromyosin and myosin induced by treatment with either guanidine or SDS²⁷ and, as shown here, can easily discriminate between atrial and ventricular myosin isoenzymes.

This possibility was first tested by an immunochemical approach, using micro-complement fixation (MCF), a technique known for its ability to detect small structural changes²⁶. Antibodies specific to a denatured structure of rat ventricular myosin were obtained by injecting guinea pigs with heavy meromyosin denatured by SDS treatment²⁷. SDS-denatured myosin from each hypertrophied heart was made to react with antisera from two different animals. There was a vertical shift in the maximum amount of complement fixed (15-35%), compared to the controls (50-70%) (Fig. 1). The immunological distances between normal and hypertrophied heart myosins were calculated by using several dilutions of antisera for the reactions²⁹. Distances were between 10 and 30, the maximum value corresponding to the myosin with the lowest enzymatic

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Table 1 Enzymatic analysis of myosins from normal and sham-operated rat hearts (S) and hypertrophied hearts (H)

Type of overloading, interval between surgery and killing (% of heart hypertrophy)	Burst size (mol ATP per myosin site)		Specific activity in steady state (nmol P _i mg ⁻¹ min ⁻¹)					
	S	H	Ca ²⁺ -activated		Mg ²⁺ -activated		K ⁺ -activated	
			S	H	S	H	S	H
(1) Aortic incompetence, 130 days (60%)	0.85	0.80	1,210	760	—	18	610	540
(2) Aortic incompetence, 141 days (93%)	0.85	0.85	1,180	860	37	27	660	690
(3) Aortic stenosis, 35 days (100%)	0.75	0.70	1,190	880	43	31	620	720
(4) Aortic stenosis, 10 days, +incompetence 92 days (90%)	0.85	0.80	1,020	730	27	18	620	760
(5) Aortic stenosis, 10 days, + incompetence 63 days (97%)	—	—	980	540	29	12	710	750

50% abdominal aorta constriction was obtained using the method of Cutilletta *et al.*¹⁵. Aortic incompetence was produced by forcing a stiff polyethylene catheter (no. 3 to 4 Biotrol) through the left carotid and then the aortic cusps. At various times after surgery, rats were killed by a blow on the head and hearts were rapidly excised. Ventricles were detached from the atria and bases of the great vessels, weighed (Vw) and frozen in liquid nitrogen within 2 min. For each animal, theoretical ventricular weight (Thw) was calculated according to the following regression curve, established in 152 controls: $\text{Thw (g)} = [(2.02 \times \text{body wt}) + 197] \times 10^{-3}$. The per cent hypertrophy was equal to $(\text{Vw} - \text{Thw}) \times 100 / \text{Thw}$. Myosin was prepared essentially according to Offer *et al.*²¹, except that ammonium sulphate fractionation was performed in the presence of 100 mM sodium pyrophosphate, 4 mM ATP and 2 mM dithiothreitol pH 7.0. The A_{280}/A_{260} ratio was between 1.4 and 1.6. Early phosphate burst size was determined manually by a slightly modified version of the method of Taylor and Weeds²³, in stoichiometric conditions²⁴, in which 10 μM myosin site (5 μM myosin) and 10 μM radioactive ATP were mixed at 22 °C, in a medium consisting of 0.5 M KCl, 50 mM Tris-HCl, 5 mM MgCl₂ and 0.1 mM dithiothreitol, pH 7.4. Steady state activities were measured at 25 °C in the presence of 2.5 mM ATP, 50 mM Tris-HCl, pH 7.5 and either 5 mM CaCl₂, 5 mM MgCl₂ or 1 M KCl and 5 mM EDTA. Inorganic phosphate was determined colorimetrically with malachite green²².

activity. The present results confirm and extend our recent observation of an antigenic difference between native myosins from normal and hypertrophied rat hearts¹⁷. It is thus unlikely that such immunological changes reflect conformational changes only. Indeed numerous observations have shown that

vertical shifts of the micro-complement fixation curves indicate amino acid substitutions²⁹.

Finally, the presence of different myosin isoenzymes in the normal and hypertrophic hearts was screened by electrophoresis of the native proteins in a non-dissociating medium. We used this technique according either to Hoh *et al.*⁹, or to a modified version of a previously described method^{30,31}. In agreement with the findings of Hoh *et al.*⁹, we observed in a highly reproducible manner three isoenzymes V₁, V₂ and V₃ (Fig. 2) in normal adult rat ventricles. V₁ had the highest mobility and was predominant in both right and left ventricles. The electrophoretic pattern was different in the ventricles from hypertrophied hearts (Fig. 2); making the reasonable assumption that the three isoenzymes were the same as in the normal hearts, a redistribution of V₁, V₂ and V₃ was found, with a predominance of V₃ in the largest and an intermediary pattern in the less affected hearts. Furthermore, the isoenzymic shift was more pronounced in the left ventricle (not shown) which is more affected in our experimental model. According to Hoh *et al.*⁹ and to our unpublished results, the specific ATPase activity of V₃ is lower than that of V₁; the increase in V₃ may therefore account for the drop in overall enzymatic activity (Table 1). These findings are consistent with the isoenzyme switches already reported between V₁ and V₃ (ref. 9). As in our model of hypertrophy, hypophysectomy induces an increase in V₃ and a fall in myosin ATPase activity,

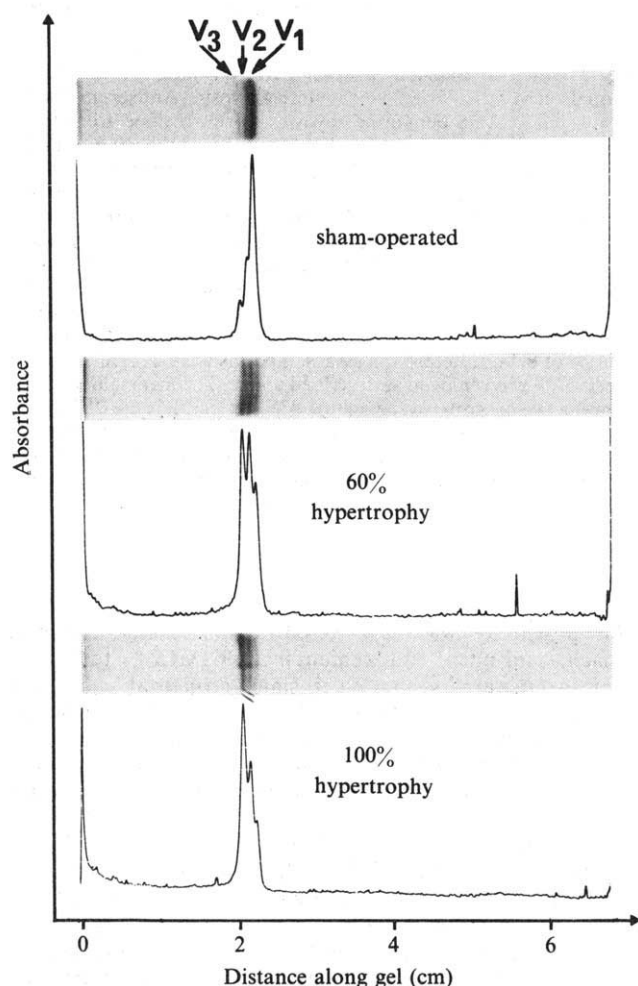


Fig. 2 Typical electrophoretic patterns and densitometric profiles for native myosins from ventricles of one normal (sham-operated) and two hypertrophied hearts (60 and 100% hypertrophy respectively). Myosin samples (1 to 3 μg) were run for 16 h on cylindrical gels (60 $\times$ 5 mm), 3.88% in acrylamide and 0.12% in *N,N'*-methylene bis-acrylamide at 14 V cm⁻¹ and 2 °C, in 20 mM sodium pyrophosphate (pH 8.8) in the presence of 10% glycerol⁹. V₁, V₂ and V₃ correspond to the three components described by Hoh *et al.*⁹. Identical patterns were observed when crude tissue extracts, prepared either with Guba-Straub's buffer or according to Hoh *et al.* where run instead of purified myosins. When run on a second pyrophosphate gel, the main isoenzyme, V₁ in the normal heart and V₃ in the hypertrophied one, migrated as a single band. The electrophoretic pattern was independent of the acrylamide concentration (2.8 to 4%). The same results were obtained in a slightly different medium consisting of 40 mM sodium pyrophosphate (pH 8.5), 1 mM EDTA and 0.01% 2-mercaptoethanol as described by d'Albis *et al.*³¹.

whereas subsequent thyroxine treatment produces both an increase of V_1 and a rise in the enzymatic activity.

It is now accepted that there is a close correlation in different types of skeletal muscles³² and in hearts of different animal species³³ between contractile element shortening capacity and myosin ATPase. Since the velocity of shortening of the cardiac muscle is reduced in gross cardiac hypertrophy induced by a mechanical overload (reviewed in ref. 2), the predominance we demonstrate in this muscle of a myosin isoenzyme of lower activity is not surprising. On the contrary thyroid hormone increases cardiac performance and more especially $V_{\max}$ (reviewed in ref. 34) and induces in hypophysectomised rats⁹ as well as in euthyroid rabbits³⁵, the synthesis of an isoenzyme of higher enzymatic activity. All these findings suggest that contractile activity of the diseased myocardium is controlled as in normal skeletal and cardiac muscles^{32,33}, by the rate of hydrolysis of ATP by its myosin isoenzyme. In addition, adaptation in cardiac performance seems to be mediated by a change in myosin species, a process which can be related to the adaptive responses of the skeletal muscles to new functional requirements¹⁰⁻¹³. The normal peak active isometric tension observed in cardiac muscles after a pressure overload³⁶ suggests that such hypertrophied muscles, in spite of their contractile defect, work with normal efficiency. Similarly, slow skeletal muscles work more efficiently when contracting at slow speeds, compared to fast muscles, and this characteristic also seems to be connected with decreased ATPase activity³⁷. The molecular basis of adaptation in cardiac performance we describe could therefore account for the compensatory step observed in chronic heart overload. It might be also hypothesised that cardiac insufficiency occurs when the adaptive change has reached its limit, that is, when the isoenzymic shift is a maximum.

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Three-dimensional structure of hyper-modified nucleoside Q located in the wobbling position of tRNA

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The hyper-modified nucleoside Q (queuosine) is exclusively located in the wobbling position of anticodons of tRNA^{Tyr}, tRNA^{His}, tRNA^{Asn} and tRNA^{Asp} that recognise codons NA₃C (ref. 1). Queuosine and its hexose-containing derivatives^{2,3} are widely distributed in microorganisms, animals and plants^{4,5}. We confirm here the chemical structure of queuosine as 7-(3, 4-*trans*-4, 5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine^{2,6} (Fig. 1). The unique structural features of Q are the unusual cyclopentenediol side chain and the 7-deazaguanine ring. Queuosine is one of the most complicated modified nucleosides found thus far⁷.

For elucidating the biological significance of this complicated modification, it is important to determine the conformation of the Q unit in the tertiary structure of tRNAs: is the conformation of the anticodon loop very different from that of usual tRNAs, such as yeast tRNA^{Phe} (refs 8-10), because of the existence of the bulky side group? Does the side chain approach the anticodon-codon interaction site and thus influence the codon recognition properties? Is the side chain buried in the anticodon loop, or exposed enough to be recognised by another molecule? To answer these questions, we first determined the three-dimensional structure of queuosine 5'-monophosphate (pQ) by X-ray crystallography. We then incorporated this conformation of Q into the anticodon of tRNA, on the basis of the

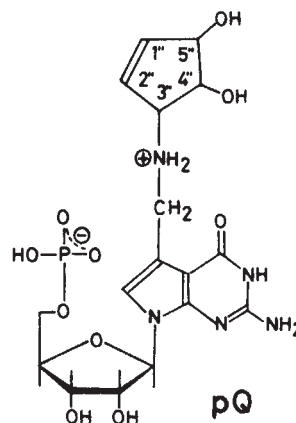


Fig. 1 Structural formula of pQ. Crystal data: 7-(3, 4-*trans*-4, 5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine 5'-monophosphate (queuosine 5'-monophosphate, pQ), C₁₇H₁₄D₁₀N₅O₁₀P·7/2D₂O, FW = 569, orthorhombic, space group P2₁2₁2₁, Z = 8, Dx = 1.528 g cm⁻³, a = 26.895(5), b = 7.707(2), c = 23.883 (10) Å, V = 4,951 Å³

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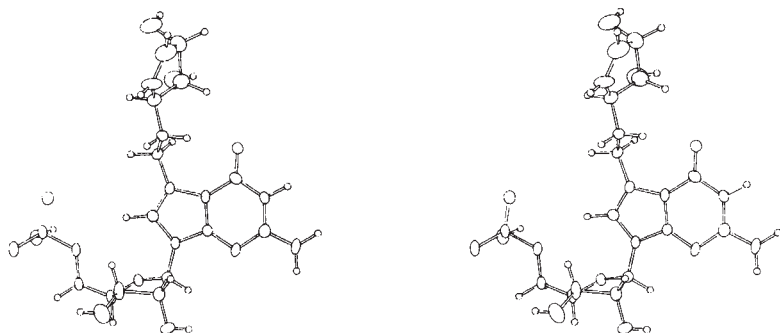


Fig. 2 Stereo view of pQ (molecule I) in crystalline form.

coordinates of yeast tRNA^{Phe} (ref. 11), as done previously for some other modified nucleosides¹²⁻¹⁴. The results indicate that the bulky 7-substituent group of Q takes the planar *trans* conformation and is fully extended outwards, so that it does not interfere with codon-anticodon interaction. Thus it seems possible that the cyclopentenediol side chain of Q may interact with other molecules in some process other than protein biosynthesis.

Queuosine 5'-monophosphate was isolated from a nuclease P₁ digest of *Escherichia coli* tRNA by Dowex 1 column chromatography¹⁵. pQ in the free acid form was crystallised from concentrated D₂O solution (*p*D, 4.5), so that the phosphate group was monoanionic and the aminomethyl group at position 7 of the 7-deazaguanine ring was positively charged. A small fragment of 0.3 × 0.08 × 1.5 mm was cut from a large crystal of 0.3 × 0.08 × 3 mm and sealed in a thin-walled glass capillary to prevent loss of water of crystallisation. The specimen was mounted on a Philips four-circle diffractometer and the unit cell dimensions and intensity data were measured with monochromated CuKα radiations using a graphite plate. The crystal data are given in Fig. 1 legend. The intensities were measured within a 2θ range of 6–120° by the θ–2θ scan method. A total of 5,589 reflections were measured as above the *I* > 2σ(*I*) level, including 965 symmetry equivalent reflections (*hkl* versus *hkl*). Deterioration of the crystal was monitored by measuring the intensities of three standard reflections. The sums of the three intensity values measured at the beginning and at the end of the measurement showed only a slight decrease (1.4%) throughout the period of measurement.

The crystal structure was solved by the direct method based on the procedure using MULTAN¹⁶. An E map calculated for the most probable set gave a plausible arrangement of peaks for phosphate groups. The whole structure was established by successive use of difference Fourier and least-squares methods. At the final stage of the refinement, water oxygen atoms were located on the difference electron density map. Four water molecules were found to occupy several sites. Their positions were assigned so that each of the four water oxygen atoms occupy with a half occupancy factor at the two separate positions not far more than 2 Å. A block-diagonal least-squared

refinement for 693 parameters of 77 atoms yielded an *R* factor of 0.117 for 4,624 observed reflections. Hydrogen atoms were not included in the refinement but they were located by model building.

The present study confirms the chemical structure of queuosine reported earlier^{2,6}. The geometries of the phosphate and imino groups indicated that the phosphate group bears one negative charge and the imino group in the side chain is protonated. Thus the molecule takes the dipolar form. The absolute configuration was determined assuming that the ribose group takes the D-configuration. It was then confirmed that the absolute configuration at C(3'') of the cyclopentene ring is the same as that determined by synthetic and CD and ORD studies¹⁷. There are two crystallographically independent molecules (I and II), which have quite similar molecular structures; the r.m.s. differences between the two molecules are 0.032 Å and 1.6° for bond lengths and bond angles, respectively, and the dihedral angles are nearly equal, being within 7° of each other. The bond lengths and bond angles of the pyrimidine moiety of the 7-deazaguanine ring are almost equal to those of the unmodified guanine ring¹⁸. It is likely, therefore, that the hydrogen-bonding or base-pairing properties of the Q base (queuine) are little influenced by replacement of the nitrogen atom by a carbon atom at position 7. The ribose ring takes the C(3')-endo form, with the exocyclic C(4')–C(5')–O(5') bond in the *g*⁺–*t* form (*ψ* = 52° and 52° and *φ* = –171° and –174°, for molecules I and II, respectively) and the glycosyl bond in the anti form (*χ* = 27° and 30°, respectively). The side chain at position 7 of the 7-deazaguanine ring is fully extended; C(7)–CH₂–NH₂⁺–C(3'')–C(2'') takes the planar *trans* conformation. An intramolecular hydrogen bond is formed between the protonated imino group and O(6) of the 7-deazaguanine ring. The dihedral angle of C(8)–C(7)–CH₂–NH₂⁺ is expected to be about 90°, as is commonly observed in related structures having a methylenic chain linked to the planar ring system. Actually however, this dihedral angle of pQ is twisted to as much as about 120°, possibly by formation of a rather strong intramolecular hydrogen bond NH₂⁺ ... O(6). The bulky 7-substituent group is extended (Fig. 2), but not towards the hydrogen bonding moiety of the Q base

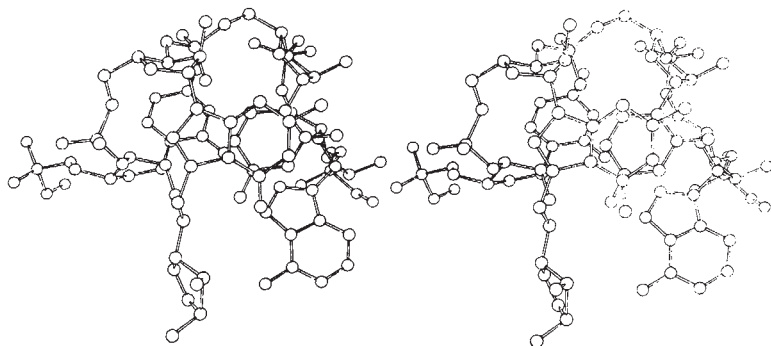


Fig. 3 Stereo view of U-Q-U-A, as constructed from the atomic coordinates of pQ (the present study) and yeast tRNA^{Phe} (ref. 11).

and the third letter of the codon (C or U). This also indicates, in accord with biochemical observations, that the codon recognition properties of Q are not significantly different from those of G (ref. 1 and S. Noguchi and S.N., unpublished). The cyclopentene ring is puckered; $C(3'')-C(2'')=C(1'')-C(5'')$ is coplanar but $C(4'')$ is displaced by 0.5 Å from this plane on the same side as the NH_2^+ group, possibly due to the existence of a diol $O(4'')H$ and $O(5'')H$ in the *cis* configuration. This puckered form is stabilised by the equatorial orientation of the large substituent $(NH_2^+-CH_2-\cdots)$.

The atomic coordinates of yeast tRNA^{Phe} (ref. 11) were used for the model-building study on the spatial arrangement of the modified nucleoside Q in the anticodon loop of tRNA. The first letter of the anticodon, Gm(34), was replaced by Q with the conformation found in the present study on the crystal of pQ. The second letter of the anticodon, adenine(35), was replaced by uracil, but the third letter, adenine(36), was retained. This sequence QUA corresponds to that in the anticodon of the tRNA^{Tyr} of *E. coli*. Figure 3 shows a stereoscopic view of the three-dimensional structure involving $U(33)-Q(34)-U(35)-A(36)$. It may be seen that Q is now incorporated into the first position of the anticodon without any steric repulsion from other parts of the anticodon loop. The cyclopentenediol group of Q is exposed out of the anticodon, in contrast to the short group of 2-thiouridine derivatives, which is more or less buried in the anticodon loop^{13,14}.

In addition to the intra-unit hydrogen bond with O(6), the NH_2^+ group of Q may also be involved in a hydrogen bonding interaction with ribose O(2') of the preceding U(33); the distance between the imino nitrogen and ribose oxygen is as short as 3 Å and $N-H\cdots O$ is nearly linear. The side group of Q(34) is thus fixed by these two hydrogen bonds, and the *cis*-diol group is orientated out to the open space, away from the base-pairing moiety of the anticodon.

The biochemical function of Q in protein biosynthesis is not yet understood. Q has a wobbling property not significantly different from that of G. Extensive studies on the properties of tRNA^{Tyr} containing G in place of Q, isolated from Q-deficient mutants of *E. coli* have not revealed any differences between Q and G with respect to binding of tRNA^{Tyr} to ribosomes or aminoacylation (S. Noguchi and S. N., unpublished). Two species of rabbit reticulocyte tRNA^{His} containing either Q or G were used with equal efficiency in haemoglobin biosynthesis¹⁹. These observations are consistent with the conformation of Q found in the present study by X-ray diffraction analysis. This hypermodified nucleoside Q is widely found in tRNAs of various organisms and thus is expected to be essential for some biological process other than protein biosynthesis. Q in *Drosophila* tRNA is suggested to have a role in cell differentiation or expression of a particular enzymatic activity^{5,20}. In tumour cells, under-modified tRNA containing guanosine in place of Q appears specifically²¹.

In conclusion, the conformational characteristics of the modified nucleoside Q appear to bring the cyclopentenediol group out of the anticodon loop, where it does not interfere with recognition of the codon of mRNA. This bulky side chain with its unique structure, charge and reactive group constitutes a suitable site for specific interaction with protein or other components. Thus the exposed cyclopentene ring (and orientation of the *cis*-diol group) of Q is possibly important for some biochemical function of tRNA other than protein biosynthesis.

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Antiparallel and parallel β -strands differ in amino acid residue preferences

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A β -strand is a particular type of extended sequence of amino acid residues, an element of secondary structure of proteins. β -sheets are an assembly of strands, often bringing together parts of the protein which are separated along the backbone. As such, β -sheets are an element of tertiary structure. Parallel (β_P) and antiparallel (β_A) arrangements of strands in a sheet differ in the hydrogen bond pattern between strands, as shown schematically in Fig. 1, and in the type of chain connectivity they allow: short reverse turn connections for β_A and longer crossover connections for β_P (refs 1-3). Most present secondary structure prediction methods (for reviews refs 4-6) use a four-state distinction of secondary structure: α -helix, β -strand or extended, reverse turn, and 'random coil' (everything else). With a data base of 30-40 different protein structures, the conformational preferences for all amino acid residues in these four states seem to have converged⁷. However, the steadily increasing data base of structurally known proteins makes a refinement of the four-state description feasible. Although more refined classifications of conformational states based on finer subdivisions of (ϕ, ψ) -space have been made^{8,9}, we prefer making distinctions based on structural environment. Using a novel definition of β -sheet structure in terms of the tertiary structure juxtaposition of strands, we have analysed residue contacts in known β -sheets and report here secondary structure preferences for the 20 amino acids, separately for antiparallel and parallel arrangements of strands. The distinction between the two arrangements results in strikingly different and sharpened sets of preference parameters, including some of the largest values reported so far for any substructure. These results point the way towards a basic improvement of secondary structure predictions by further distinction of secondary structure elements according to tertiary structure environment. Beyond secondary structure prediction, the different preferences for β_A and β_P may aid in predicting the tertiary interaction between strands.

Our independent compilation of β_A - and β_P -residues in 30 proteins (a list of the proteins is given in ref. 10) defines β -sheets by their strand-strand interactions, rather than by single residue conformation, such as backbone (ϕ, ψ) angles. The protein coordinates are from ref. 11 (and updates as of August 1978). We identify two neighbouring antiparallel (or parallel) stretches of polypeptide chain (at least three residues each) as a pair of β_A (or β_P) strands (1) if pairs (i, j) of contacting residues have their side groups on the same side of the sheet (Fig. 1), and (2) if the $C=O$ and $N-H$ groups of the backbone are properly orientated¹⁰. Single strands are not counted. For regular stretches of

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Table 1 Amino acid residues in antiparallel and parallel β -strands

	Residue contact count			Frequency (%)				Conformational preference [‡]							
	β_A	β_P	All β	β_A	β_P	All β	Global*	β_A	β_P	β_P/β_A	All β :	Here	Ref. 13	Ref. 14	Ref. 12
Gly	81	38	119	5.1	7.2	5.7	9.2	0.56	0.79	1.41		0.61	0.75	0.66	0.92
Pro	29	8	37	1.8	1.5	1.8	4.4	0.42	0.35	0.83		0.40	0.55	0.84	0.64
Asp	39	14	53	2.5	2.7	2.5	5.3	0.47	0.50	1.08		0.48	0.54	0.64	0.72
Glu	47	15	62	3.0	2.9	2.9	4.8	0.62	0.59	0.96		0.61	0.37	0.61	0.75
Ala	119	44	163	7.6	8.4	7.8	8.4	0.90	1.00	1.11		0.92	0.83	0.79	0.90
Asn	41	12	53	2.6	2.3	2.5	4.2	0.62	0.54	0.88		0.60	0.89	0.66	0.76
Gln	63	5	68	4.0	1.0	3.2	3.4	1.18	0.28	0.24		0.95	1.10	1.13	0.80
Ser	123	33	156	7.8	6.3	7.4	9.0	0.87	0.70	0.80		0.82	0.75	0.84	0.95
Thr	139	21	160	8.8	4.0	7.6	6.8	1.30	0.59	0.45		1.12	1.19	1.14	1.21
Lys	83	22	105	5.3	4.2	5.0	7.1	0.74	0.59	0.79		0.70	0.74	0.72	0.77
Arg	45	10	55	2.9	1.9	2.6	2.8	1.02	0.68	0.67		0.93	0.93	1.04	0.99
His	44	5	49	2.8	1.0	2.3	2.5	1.12	0.38	0.34		0.93	0.87	0.78	1.08
Val	188	108	296	11.9	20.5	14.1	7.8	1.53	2.63	1.72		1.81	1.70	1.97	1.49
Ile	112	63	175	7.1	12.0	8.3	4.6	1.54	2.60	1.69		1.81	1.60	1.95	1.45
Met	24	11	35	1.5	2.1	1.7	1.4	1.09	1.49	1.37		1.19	1.05	1.26	0.97
Cys	45	11	56	2.9	2.1	2.7	2.3	1.24	0.91	0.73		1.16	1.19	1.55	0.74
Leu	141	53	194	8.9	10.1	9.2	7.1	1.26	1.42	1.13		1.30	1.30	1.26	1.02
Phe	68	24	92	4.3	4.6	4.4	3.5	1.23	1.30	1.06		1.25	1.38	1.30	1.32
Tyr	98	21	119	6.2	4.0	5.7	3.7	1.68	1.08	0.64		1.53	1.47	1.49	1.25
Trp	47	8	55	3.0	1.5	2.6	1.7	1.75	0.89	0.51		1.54	1.37	0.90	1.14

The order of amino acids reflects their average structural similarity¹⁷. Amino acids which are observed to be more mutually exchangeable in evolution are nearer each other in this list.

* Numbers taken from ref. 11.

[†] The preference for $\beta_A(\beta_P)$ is a normalised frequency: the frequency in $\beta_A(\beta_P)$ divided by the global frequency. Note that the definitions of β -residues vary among different authors^{7,13,15}. Furthermore, our preferences are for making β -sheet contacts, whereas those of the other authors are for occurrence in extended or β -strands.

β -strands (generally away from the edges of sheets) our identification of β -residues agrees with that of the crystallographers. As we define β -residues by their contact with neighbouring strands, we count residue contacts rather than residues in deriving the single residue frequencies. Thus, residues in strands at the edge of a sheet are counted once, residues in interior strands are counted twice, and residues making β_A contact on one side and β_P on the other are counted once for each type of contact.

The residue contact counts and residue contact frequencies are given in Table 1. β_A structures outweigh β_P by about three to one in the data base. The amino acid compositions of β_A and β_P are remarkably distinct. About one-third of all residue contacts in β_P are made by Val and Ile, the two non-polar side chains branched at C_β . Val (21%), Ile (12%), Leu (10%) and Ala (8%), with purely aliphatic side chains, together account for more than 50% of all contacts in β_P . In β_A six amino acids are needed to reach the 50% mark, including the polar side chains Thr and Ser, and the order of abundance (listed in decreasing order) is different: Val, Leu, Thr, Ser, Ala, Ile.

The 'conformational preference' parameters presented in Table 1 are defined as the ratios between the frequency of pair-contacts made by a residue in β -strands and the global

frequency of occurrence of that residue anywhere. Values larger than 1.0 indicate preference of the particular residue for $\beta_A(\beta_P)$, values less than 1.0 indicate the opposite. Conformational preference parameters (derived from residue frequencies rather than from contact frequencies) have been used by many authors (for reviews see refs 4–6, 12) in the prediction of secondary structure (called 'potential', 'propensity', 'information measure'). The parameters given by Chou and Fasman¹³, by Garnier *et al.*¹⁴, and by Levitt¹² are included in Table 1 for comparison. There is overall agreement between the 'all β ' (β_A and β_P together) preferences in the different studies. The differences, for example, for Pro, Glu or Trp, arise because the studies use not only different sets of proteins as their data base, but also different ways of identifying β -residues^{7,13,15} and, in our case, different weights for interior and edge strands.

Comparison of conformational preferences between β_A (antiparallel) and β_P (parallel) shows: (1) the β_P structure is more selective: there are more extreme large and small values in β_P (standard deviation 0.66 for β_P but only 0.40 for β_A ; high and low: 2.63 and 0.28 for β_P but only 1.75 and 0.42 for β_A), and, in β_P fewer residues are preferred (only 6, compared with 12 in β_A) and for these few the preference is stronger (except Tyr). In fact, the 20 amino acids can be divided into three groups (Table

Table 2 Conformational classification of β -sheet residues

		Conformational preference
Favourable in both β_A and β_P	Val Ile Met Leu Phe Tyr	>1.0 in β_A and β_P
Favourable in β_A but unfavourable in β_P	Gln Thr Arg His Cys Trp	>1.0 in β_A and <1.0 in β_P
Unfavourable in both β_A and β_P	Gly Pro Asp Glu Ala Asn Ser Lys	≤ 1.0 in β_A and β_P
Best sheet 'makers' in β_A	Trp > Tyr > Val > Ile	>1.5
in β_P	Val > Ile > Met	
Worst sheet 'breakers' in β_A	Pro < Asp < Gly < Glu < Asn	$\leq 1/1.5$
in β_P	Gln < Pro < His < Asp < Glu < Thr < Lys	

2)—favourable in both β_P and β_A , favourable in β_A but not in β_P , and unfavourable in both β_A and β_P . (2) β_P 'abhors' polar and charged groups and favours hydrophobic side chains more strongly than β_A : only the purely hydrophobic residues Val, Ile, Leu, Met, Phe are significantly preferred (>1.2) in β_P , whereas in β_A also Thr, Cys, Tyr and Trp, all containing polar atoms, show values above 1.2. (3) Val and Ile are particularly favoured in β_P , with the second largest secondary structure enhancement values reported so far—2.6. Only the value for Pro at the second position in reverse turns¹⁶ is larger—3.4. In β_A the preferences for Tyr and Trp (1.7) are exceptionally large. Note that both have a large non-polar ring in combination with a polar group. (4) The differences between β_A and β_P are particularly large for Gln, Thr, His and Trp (favourable in β_A and unfavourable in β_P); Gln is less favoured in β_P by about a factor of 4.

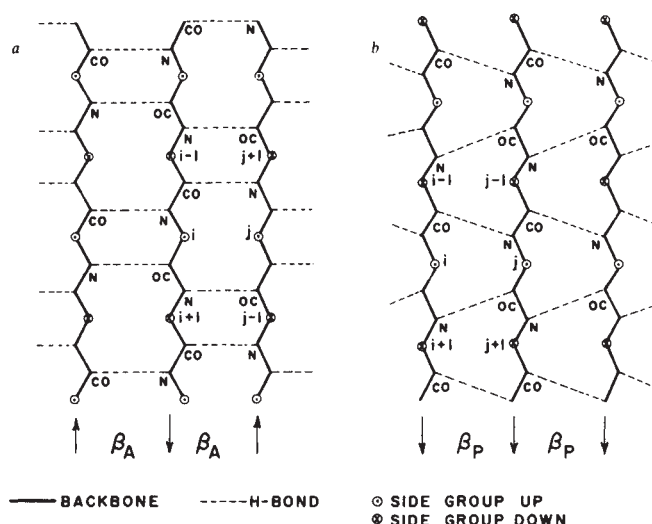


Fig. 1 A schematic drawing of antiparallel (a) and parallel (b) β -strands. The arrows indicate the N-terminal to C-terminal direction of the polypeptide backbone. Although the (ϕ , ψ) backbone angles are similar for β_A and β_P , the arrangement of the strands and the hydrogen-bonding patterns are fundamentally different. This difference is also reflected in different amino acid preferences.

The results presented here are potentially useful in two ways. On the one hand, they help to pose more specific questions, by focusing attention on those residues which have a special structural role. In this, residues which are both abundant and preferred are the most interesting. For example, one may ask: can Val and Ile serve as nucleation points for β_P -strands by restricting the backbone conformation with their side chains? Do they pack particularly well in the environment of the crossover connection between β_P -strands? Do they interact preferably with each other in forming clusters on the surface of β -sheets? The aim in investigating this type of question is to understand the physical basis of the statistical preferences.

On the other hand, the conformational preferences can be incorporated into structure prediction schemes, allowing distinct predictions for antiparallel and parallel β -stands. However, when this is done the limitations inherent in all statistical approaches to protein structure must be kept in mind: proteins are biologically evolved molecules with a particular structure tailored to a particular function. Only average properties of protein structures can be understood by statistical methods, but not the highly individual character of each protein.

Thus, the main conclusions regarding protein architecture are: (1) antiparallel and parallel β -strands are as distinct in their amino acid preferences as are α -helices and β -strands; (2) parallel β -strands impose much more severe constraints on their amino acid content than antiparallel β -strands or α -helices; (3) Val and Ile have an outstanding role in parallel strands. In a related paper¹⁰, we analyse interstrand pair recognition of amino acid residues and its role in the tertiary structure assembly of strands into sheets.

As more protein structure data become available, further distinctions of secondary structure elements according to the type of tertiary contacts should be made. For example, one can distinguish different hydrogen-bonding positions in β -sheets, solvent-exposed and interior faces of sheets or helices, segments in tertiary contact with sheets compared with those in contact with helices. Such distinctions are likely to lead to more clearcut statistical preferences, and also serve as a starting point for predicting tertiary structure.

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Errata

The letter 'Local destabilisation of a DNA double helix by a T–T wobble pair', *Nature* **281**, 235, was printed with errors in the author line. This line should read:

A. G. Cornelis Haasnoot, Jeroen H. J. den Hartog, Jan F. M. de Rooij, Jacques H. van Boom & Cornelis Altona.

In the paper 'Isolation, cloning and sequence analysis of the cDNA for the α -subunit of the human chorionic gonadotropin', *Nature* **281**, 351, figures 1 and 2 should be transposed. The legends remain as published.

In the paper 'Unmasking of fetal determinants on adult bone marrow cells', *Nature* **281**, 484, the second author should be spelt B. M. Sheinberg.

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matters arising

Evidence for a higher natural uranium content in world rivers

THE letter by Mangini *et al.*¹ on aspects of the geochemical cycle of uranium requires comment. They attempt to show that phosphatic fertilisers do not contribute significantly to the uranium budget of rivers or groundwater, but the arguments and evidence they produce to support this claim are deficient.

They claim, from the observed phenomenon that rivers show a significant ^{234}U excess but that phosphate fertilisers do not, a contradiction to the assumption of a pollution effect. Yet the ^{234}U excess merely reflects the preferential leaching of this daughter product rather than its parent ^{238}U . Since this fractionation was noted in 1953² it has been developed as a uranium exploration tool³ and a dynamic equilibrium model⁴ has been developed showing its variation about source rocks which themselves have an average $\text{AU} \sim 1$ (AU , ratio of ^{234}U to ^{238}U).

A similar claim is made that the close relationship found by my colleagues between uranium content and bicarbonate (HCO_3^-) content⁵ for rivers and groundwaters indicates a natural origin for the uranium. Again, this relationship merely shows the common mode of migration of uranium in solution as a bicarbonate complex and gives no evidence for the source of the uranium.

The lack of a clearcut relationship between the uranium and phosphate contents of the water is further taken as evidence for a negligible contribution of uranium from phosphatic fertilisers. Again this is a function of the relative solubilities of the uranium and phosphate components and itself provides no evidence for the source of the uranium. In the Devonian sediments of northern Scotland there are naturally occurring bedded concentrations of uranium in phosphatic rocks^{6,7} and related uranium anomalies are present in the surface water, but these exhibit no corresponding anomalies in phosphate content. There is thus selective leaching of uranium from these bedded phosphatic sediments.

Additionally, both in the Caithness and Orkney areas a limited number of spuriously high values were detected (>10 to $100 \mu\text{g l}^{-1} \text{U}$) and these were related to transient enrichments from fertilised areas. Samples of phosphate fertilisers used in the area contain up to $100 \mu\text{g U}$ per g. This was taken as supportive evidence for the regional scale contamination noted by Spalding and Sackett⁸, but rejected by Mangini *et al.*¹.

Rock phosphate (apatite/collophane) is able to fix uranium in both oxidising and reducing conditions. Uranium can be present both in the U^{4+} form, replacing Ca^{2+} in the apatite structure, and as U^{6+} where it is adsorbed on the surface. It is the adsorbed uranium which is readily removed by surface waters. Experimentally Spalding and Sackett⁸ showed the ease with which uranium may be leached by water from superphosphate fertiliser. They also demonstrated that after application of fertiliser and 0.5 inches of rainfall equivalent, the uranium level in the run-off was 28 times greater than in a similar blank experiment.

Mangini *et al.*¹ present groundwater evidence from a highly fertilised area which does not show any enhancement of uranium in the subsurface waters and take this to indicate that "more than 95% (2σ) of uranium supplied with the fertilisers is retained in the uppermost soil layers". They also note that "less than 0.01% of the phosphate goes into groundwater" [I presume ND in their Table 1 means 'not detectable' and not 'not determined' as stated] and state "assuming a similar transport mechanism for uranium the contamination would make less than $0.004 \mu\text{g l}^{-1}$ ". As indicated in the preceding paragraphs this assumption must be rejected as uranium is leached separately from the phosphate.

The explanation for the low uranium values in the groundwater, although the added fertiliser contains 50 p.p.m. U, may simply be that the uranium is adsorbed and complexed from the groundwater in the soil profile but could still be being leached by the surface run-off. No figures for uranium in the surface run-off are presented and, as the area has been fertilised for several decades, the original background may not be determinable. A transient increase following application of fertiliser should be sought.

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MANGINI REPLIES—Our conclusion that in the Handschusheimer Feld area the influence of uranium from phosphate

fertilisers is small was derived both from the low uranium contents of groundwater and river water, more importantly, from their high AU (~ 1.6).

We appreciate the opportunity to clarify a point to those not familiar with ^{234}U and activity ratios (AU). ^{234}U is a member of the ^{238}U decay series, with a half life of 244,000 yr. Disequilibrium between ^{234}U and ^{238}U ($\text{AU} \neq 1$) is a common phenomenon in nature. Rocks and clays from which uranium is being extracted have $\text{AU} \leq 1$, rivers and groundwaters $\text{AU} > 1$ (average 1.20) and recent uranium deposits (younger than 10^6 yr) $\text{AU} \geq 1$. In uranium-enriched phosphate fertilisers we find, as expected, $\text{AU} \sim 1$.

Preferential selective leaching of ^{234}U from rocks and clays is attributed to the α -recoil on the ^{234}Th nucleus, following the ^{238}U α -decay. Recoil energies of approximately 0.1 MeV 'shoot' ^{234}Th (the short-lived grandmother of ^{234}U) from a 550-Å thick surface layer of the mineral grains into solution¹. Whenever traces of uranium are leached, the solution will have $\text{AU} \geq 1$.

The situation is different in the upper soil layers, where uranium is being supplied by fertilisation at $\text{AU} \sim 1$.

In fact a competitive fertiliser should nearly completely dissolve in a period of months. The consequence is that nearly all uranium (^{234}U and ^{238}U) undergoes dissolution (as confirmed by Spalding and Sackett's experiments) and, we believe, will be adsorbed and complexed in the uppermost soil layers— ^{234}U in the same chemical form as ^{238}U . If, either by groundwater or surface run-off from this soil, any leaching of uranium occurs, dissolved uranium should have the same AU (~ 1) as the soil, as there is no chemical difference between complexed ^{234}U and ^{238}U . Even after, say 1,000 yr, less than 3% of ^{234}U will be the more easily leachable 'recoil ^{234}U '.

Tritium measurements on precipitation and stream water in the Neckar system indicate that, for heavy precipitation, surface run-off contributes less than 5% to the river discharge. We presume that most of this run-off contribution originates in the inclined areas of the river spring system where horticulture should be negligible².

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reviews

Born's intimate reminiscences

P.S. Farago

My Life: Recollections of a Nobel Laureate. By Max Born. Pp.308. (Taylor and Francis: London; Scribners: New York, 1978.) £8.50; \$17.50.

THE basic facts about Max Born's career can readily be found; there is an entry under his name in every respectable encyclopaedia. One aspect or another of his pioneering work is well known to any competent physicist; many received their first introduction to atomic and molecular phenomena from his *Atomic Physics*. Born himself wrote several retrospective essays, some of which are collected in the volume *My Life and My Views*; and an excellent study of his life and works appeared in 1971 in the Biographical Memoirs of the Fellows of The Royal Society. Yet there has been relatively little information about the forces which shaped his character and outlook, the motives which turned him from an average school-boy into a physicist of the greatest distinction, or his relations with the contemporary academic community and society at large. These and many similar questions are answered in this volume.

Max Born had just turned 50 and was at the height of his career when Hitler rose to power, and a few months later he was deprived of his position. He writes: "All I had built up in Göttingen, during twelve years' hard work, was shattered. It seemed to me like the end of the world". And in a way it was. He left Germany, and during his 20 years of exile he found no true spiritual home. After three years of limited security in a Cambridge lectureship he was appointed to the Tait Chair of Natural Philosophy at the University of Edinburgh in 1936. He stayed there until his retirement at the age of 70. The major part of the recollections, under the title "Good Old Days", was written here during the War. It takes the story up to 1925, including the birth of Quantum Mechanics. The second part, entitled "Tempestuous Years", is a more sketchy account of the rest of his active life, written in retirement in Germany when he was about 79. The Preface by his son tells us that these memoirs were written primarily for his family. Indeed the reader feels as if he were admitted to this intimate circle to witness Born re-living his eventful life. The narrative is loosely spun of several strands and presented in a discursive style.

Firstly, the story of his family. On his mother's side, rich and influential industrialists enjoying a life of luxury,

highly educated amateurs and patrons of the arts (mainly music and literature), friends of and hosts to eminent artists of the time. "My father's ancestry", he writes, "was humbler in regard to all things of property and consequence in the world, but I believe that the essence of my nature is due to the Born clan". As the narrative unfolds there emerges a view of the social



Max Born in 1926

and political scene in Prussia about the turn of the century, and in Germany as a whole during the following decades.

Against this back-drop one can see the working of the factors which shaped his remarkable character. A fixed reference frame was defined for him by the traditions of his Jewish ancestors, emancipated generations earlier. "They believed they differed from their neighbours only in respect of religion, for which they had hardly any use, and they were Germans by culture and custom". This deep-seated sense of identity, an "inextinguishable homesickness for the German language and landscape", played an important part in deciding him to return to Germany after his retirement.

Born's concept of right and wrong began to be formed at a tender age and crystallised early, under his father's guidance. He recalls touching examples of his father's methods of teaching "practical ethics". The moral code thus assimilated did not conflict in any way with the enjoyment of beauty in nature and in art, and with taking a fair share of all the pleasures of a full life.

Intertwined with the family saga there appears a vivid picture of the contemporary educational system, and of academic life with its social and

professional facets, depicted first from the point of view of a student, and then as seen by a scientist of rapidly growing stature. Fascinating portrait sketches bring to life many unique individuals, his teachers, fellow students, eminent colleagues of several generations, and highly gifted pupils. Some are portrayed with affection and respect, some observed with critical eyes and some drawn in the style of mocking cartoons.

As the narrative progresses he overcomes his apparent reluctance to 'talk shop'. Bit by bit there emerges a complete, though non-technical, survey of his own work and his own assessment of its significance. Reading these passages one can catch a glimpse of the way his creative intellect worked and of the emotional response to recognition given or denied to him at various stages of his career. Some of his attainments he thought were overrated, others were not appreciated adequately. Above all, the lack of recognition for his contribution to the foundation of the principles of Quantum Mechanics left a very deep and long-lasting scar, healed only by the Nobel prize 28 years later, during his retirement.

Max Born and his contributions to our knowledge of the physical world became enduring features of the history of science. This volume of his reminiscences reveals a whole world unknown to the present generation, and through it one can piece together the complex personality of the narrator. His reflections on a philosophical seminar he attended as a student in Göttingen could stand as his testament: "I can quite well grasp Kant's idea that there are principles or categories of thinking which are the conditions of actual knowledge and which you can discover by investigating the structure of this knowledge. That is what we theoretical physicists are really doing — with the sole difference that we do not claim that our latest analysis is a final and irremovable law, but just another step to a remote truth. To look for ultimate evidence by introspection, contemplation and verbal analysis, is irreconcilable with science, and seems to me in practical life the source of all ideological struggle. For anyone who believes he has obtained such an internal evidence easily becomes a fanatic, a mystical believer unapproachable by reason and argument". □

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Parasite physiology

Biochemistry and Physiology of Endoparasites. By Theodor von Brand. Pp. 447. (Elsevier/North Holland/Biomedical: Amsterdam; New York and Amsterdam, 1979.) \$87.75; Dfl.180.

THEODOR VON BRAND died in 1978 and parasitology lost the man who had pioneered the study of the physiology and biochemistry of parasites and whose name had become synonymous with the subject for almost forty years. von Brand was probably the best person to be able to overview the whole of this area of study and fortunately he managed to complete the text of this book before his death. This remarkable synthesis of a massive amount of information will remain as a tribute to the man himself and will join his previous works, *Chemical Physiology of Endoparasitic Animals and Biochemistry of Parasites*, two of the most influential books ever written on parasitology.

Biochemistry and Physiology of Endoparasites is an updated version of *Parasitenphysiologie* published in German in 1972. There are ten chapters each with between 170 and 550 references mainly from the 1960s and 1970s. The chapters include the obvious ones on nutrition, respiration composition, synthetic processes, degradation processes, and sensory, nervous, muscular and locomotory physiology, and less obvious ones on parasitic habitats, hormones, pathophysiology and the physiological basis of chemotherapy. There is therefore something of interest for everyone. Von Brand wrote beautifully and was also a master of synthesis: the essentials of whole papers are expertly digested and summarised in a few words so carefully chosen that anyone can understand them. This is a book for casual reading and for reference but it is as the former that it will be most useful. The committed parasite physiologist will have access to specialist reviews and literature retrieval facilities but even he should be able to use this book to put his work into some sort of general context.

A book of this kind cannot, of course, please everybody and it is stronger on helminthology than protozoology, physiology than biochemistry, and physiology proper than the rather newer fields of pathology, immunology and chemotherapy. This uneven coverage is inevitable and probably reflects real differences in our depth of knowledge of various aspects of parasitology.

It is customary today to criticise the price of books and in this case the publishers have done a considerable disservice to parasitology, which by its very nature is a subject of particular importance in the poorer parts of the world. The price will

limit the purchase of this book to specialists who, for reasons given above, will learn least from it. This is a pity because this book summarises the state of the art; and as this subject is expanding so rapidly the preparation of a revised edition will be an impossible task. It is to be hoped that the publishers will find it possible to produce a cheap version for parasitologists in the tropics and students.

Had this been a cheap book the mistakes in it could have been forgiven but there are too many of them and they do mar the book and reduce its value as a work of reference. For example, there are inconsistencies in the spelling of names (Bernstein and Baernstein, Buelbring and Bülbring); the sources of figures and diagrams are given but do not appear

among the references (Smyth (1969) on page 196 and Ryley (1967) pages 184-185); the names of parasites are not given in the captions to some of the figures (Figs. 41 and 49); and worst of all, and proof that these mistakes are not those of von Brand himself, his name appears under the Bs in the references to chapters 1 and 2 and the Vs elsewhere.

These shortcomings, however, are trivial compared with the importance of this book as a whole. No-one else will ever write a book of this kind and no parasitologist should fail to read it.

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Reichenbach's writings

Selected Writings, 1909-1953. Two volumes. By Hans Reichenbach. Principal translations by Elizabeth Hughes Schneewind. Edited by M. Reichenbach and R. S. Cohen. Pp. 500 + 435. (D. Reidel: Dordrecht, The Netherlands, Boston, and London, 1979.) Vol. 1: hardback Dfl. 145, \$64.50; paperback Dfl.60, \$27; Vol. 2: Dfl. 130, \$59 and Dfl.60, \$27.

HAD Hans Reichenbach (1891-1953) not died unexpectedly at the age of 62, a volume on him in the Library of Living Philosophers would have certainly gone beyond the stage of preliminary planning. According to the structure of volumes in that Library, an autobiography of Reichenbach would have been followed by a series of essays by other philosophers on the various aspects of his thought and a reply by him in addition to a list of his publications. This two-volume set contains such a list and starts with an autobiographical sketch followed by over thirty reminiscences by members of his family, friends, colleagues, and students which cover his childhood, university years, his first teaching post in Stuttgart's Technische Hochschule, his tenure at the University of Berlin, his stay from 1933 until 1938 at the University of Istanbul, and finally his years as a widely acclaimed professor of philosophy at the University of California in Los Angeles and a chief spokesman of logical positivism in the United States.

These reminiscences are full of illuminating gems about Reichenbach's life, work, thought, and personality which only the eyes of admirers could discern and retain for posterity. Anyone nurturing the notion that the Vienna Circle, of which Reichenbach was a prime mover as the founder of its organ, *Erkenntnis*, was given entirely to abstractions, can now learn

from a most reliable source that Reichenbach considered himself a crusader. On hearing himself described as one whose sole interest was logic and epistemology, he reacted angrily: "But no! That is not true! The whole movement of scientific philosophy is a crusade . . . what I am doing aims as directly at social consequences as the programs of those who call themselves social reformers". The hitherto untranslated or no longer in-print publications of Reichenbach, which make up the bulk of these two volumes, have as their first group eight essays on social problems, written before and during World War I, with a strongly Socialist though not Marxist accent.

Equally instructive is Reichenbach's characterisation of the indispensability of philosophers: they (he means above all himself — witness his *The Rise of Scientific Philosophy* in which the modern part is largely limited to his own philosophy) are alone capable of articulating what the scientists, more often than not, brilliantly do but clumsily say. Reichenbach was a master of articulating not only the most abstract scientific notions and procedures (he had his own programme at the State Radio in Berlin), but also of handling down-to-earth matters. An engineer by training (he did not want to become an engineer "who can do nothing but calculate") he was competent in household repairs. He could deliver impromptu speeches on most obtruse topics, had touching sensitivity for others' needs, did pioneering work in interdisciplinary teaching, but, as stated in one of the reminiscences, was possessed "like most philosophers . . . of a strong sense of vanity".

The pride and centre of Reichenbach's philosophy was the probability theory of existence. He came to it partly through his early fascination with the kinetic theory of gases, partly under the impact of quantum theory, and partly because he took Einstein's relativity as a proof that questions about reality as such were meaningless. It was through Einstein's

theories of space and time that Reichenbach perceived the errors of Kantian metaphysics.

The items offered in Part II and Part III under the respective titles "Popular Scientific Articles" (21 items published between 1922 and 1932) and "General Scientific Articles" (11 items of which 10 were published between 1925 and 1933) give much insight into both Reichenbach's effort to develop a "scientific" philosophy, whose sole criterion of truth was physics, and into his keen interest in the latest developments of science.

Reichenbach is always his own voice even when he speaks of others, a desirable stance when taken by a great mind, but also rich in unintended instructiveness. The best example of this is provided in Reichenbach's writings on Einstein, his hero, whom he knew at close range, as he had attended with four others Einstein's first seminar in Berlin. There is no trace in these two volumes — judiciously selected, brilliantly translated, carefully edited and annotated — that Reichenbach had ever taken heed of the increasingly more emphatic endorsements by Einstein of a metaphysically realist epistemology and of his summary dismissals of positivism, including its "logical" brand.

The historian of science will be surprised on finding that Reichenbach never makes as much as a modest effort to take a critical look at inherited clichés relating to scientific history (his reading of Kant's early scientific writings is very superficial indeed) and to live up to his professed premise to be respectful only of carefully established facts. Though largely uninterested in history, he did not, unlike some others in the Vienna Circle, forge facts (to recall a famous 'confession' of Feigl) to suit theories.

Two articles on ethics and free will, which as Part IV complete Volume I, are for all their brilliance in argumentation a perfect case of myopia concerning the circularity in which Reichenbach's classification of moral judgments as volitional decisions is caught. Is not Reichenbach's own decisional definition of cognitive meaning the very basis of that classification?

Volume II, which contains Part V ("Philosophy of Physics") and Part VI ("Probability and Induction"), is harder reading, but the gleanings are rich both for admirers and critics. The question of "now" as handled by Reichenbach in an essay of 1925 is a case in point. The existential, irreducible aspect of "now" never comes within his ken. The sole alternatives for him are choices between mathematical techniques, which he handles superbly, appropriate for deterministic and probability sequences, respectively. It was, of course, much later that Einstein reminded Carnap about "now" as something which slips through the nets of physics, but not so late that Reichenbach, a close friend of Carnap,

could not have heard of it.

The most notable item in Volume II is a translation, under the title "The Aims and Methods of Physical Knowledge," of an essay originally published in 1929 in the section of the famed *Handbuch der Physik* dealing with the general foundations of physics. Reichenbach was obviously well known and highly regarded in the Germany of physicists, then the home of many major advances in theoretical physics. The very title of that essay cannot help remind one of a classic book of Duhem which was well known in German translation in the Vienna Circle. In the more than a hundred footnotes and bibliographical references of that essay, Duhem's name occurs not once, nor in the two volumes for that matter, a telltale sign of the narrowness which logical positivism can generate.

Analytical flame spectroscopy

Fundamentals of Analytical Flame Spectroscopy. By C. Th. J. Alkemade and R. Herrmann. Pp. 442. (Adam Hilger: Bristol, UK, 1979.) £25.

THIS book makes an extremely good and successful attempt to treat the three branches of analytical flame spectroscopy — atomic emission, absorption and fluorescence — in a unified way. It provides the novice and aficionado alike with a sure guide to the understanding of the phenomena involved in atom production within chemical flames and their subsequent excitation for emission, absorption or fluorescence.

There are many books that treat analytical flame spectroscopy, but this one is probably unique in that it deals almost exclusively with the fundamental aspects of flame spectroscopy as they relate to practical analysis. Early on, the authors make the point that, although it is relatively easy to establish an analytical method based on atomic spectroscopy for the determination of a given trace element under defined circumstances, it is not so easy to extrapolate it to other conditions or to explain interferences and limitations. It is also difficult usually to establish successful adaptations without a knowledge of the fundamental processes that are involved. To this end the text incorporates the most relevant results of fundamental studies in atomic spectroscopy, the physics of collisions, chemical kinetics, combustion research, and so on. There are chapters on flame structure, pneumatic nebulisation, desolvation and volatilisation of analyte species, dissociation and ionisation, background emission and absorption, shapes of analytical growth curves, and the classification of the interferences of con-

The last item in Volume II is a letter which Reichenbach wrote on March 28, 1949, to Bertrand Russell. Reichenbach hoped that a discussion of an hour or two would settle the dispute between them and then, as Reichenbach put it, "you would see that your abandonment of empiricism is unnecessary and that you need not resort to an extra-logical principle not based on experience". A vain hope, indeed. Russell had since long achieved a better grasp of the limitations of logic and method than the best which Reichenbach could ever muster.

Stanley L. Jaki

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comitant substances. Throughout this treatment the three branches are dealt with synoptically or in juxtaposition so that their relative merits or limitations stand out clearly. There are also numerous references to the corresponding phenomena in electrothermal atomisation, thus enabling the reader to make his own assessment of the alternative (non-flame) atomisation technique.

The level of treatment assumes a basic knowledge of physics and chemistry that is possessed by most graduate students or experienced analysts who use atomic spectroscopy; it should not cause many problems to the average reader in this respect. It is also a matter of considerable satisfaction to find the authors using the terminology and nomenclature recommended by the Spectrochemistry Commission of the Analytical Division of the International Union of Pure and Applied Chemistry. These terms are defined in a glossary which reproduces the IUPAC report and, additionally, cross-references the earlier terms which abound in the literature. This section comprises 223 pages and is followed by a useful list of flame spectrograms showing the emission spectra of flames, hollow cathode lamps and atoms in flames. An index of flame spectra (lines and bands) is provided for the range 177.495–1252.2 nm to facilitate the identification of unknown peaks; and, finally, there is a comprehensive list of flame spectra arranged in alphabetical order of the elemental symbol. Over 900 references are cited and there is a comprehensive subject-author index.

There is no doubt that this is an extremely valuable book for background reading and general information for all who use analytical atomic spectroscopy in flames.

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Comprehensive survey of the pulmonates

Pulmonates. Volumes 2A and 2B. Edited by V. Fretter and J. Peake. Pp. 540 and 150. (Academic: London, and New York, 1979.) £24.50, \$50.75 and £12, \$25.20.

ANIMALS with high potential for colonising the land came into existence with the first appearance of the Gastropoda, of which the pulmonates form a subclass. The compact body was protected by an impervious shell with a restricted opening into which the broad foot was withdrawn; and the enclosed mantle cavity easily converted into a respiratory sac. Emancipation of the reproductive from the renal system made internal fertilisation possible and removed the last barrier to landward passage.

The subclass Pulmonata is classified into two orders. The modern Stylommatophora possess unique ingenuity in resisting the prime danger of dessication; and it is a rare terrain that is devoid of these animals capable of remaining dormant for the greater part of the year, even for years on end. In contrast the Basommatophora have returned to aquatic life — largely in freshwater, but in certain seas they are amongst the commonest intertidal limpets.

Dependence by the majority of snails and slugs on plant life has made them major competitors with man for food; and their association with other animals, notably in damp places, has made them admirable intermediate hosts for parasites. They are thus animals of high economic significance and for that reason also worthy of careful study.

These volumes represent the completion of a comprehensive survey begun in 1975 with the publication of a volume dealing with functional anatomy and physiology. In volume 2A treatment covers *systematics, evolution and ecology* presented in 10 chapters by highly competent authors.

With unique wealth of experience, B. Hubendick reviews systematics and comparative morphology in the Basommatophora revealing the range of structural variety from high spired snails to flattened limpets. This is followed by consideration from the equally authoritative pen of A. Solem of the data on which classification of the 20,500 odd species of land molluscs, almost entirely Stylommatophora, is based. In both chapters, unfortunately, references are largely restricted to those before 1972.

G.M. Davis, concerned with precise demarcation of species where transmission of human parasites is involved, reveals the importance of the new methods of chromatography, electrophoresis and immunology. This leads on to an account

by C.M. Patterson and J.B. Burch of the results of work on the chromosomes of pulmonate molluscs. Chromosomes vary from 5 to over 70 pairs although variation is usually conservative within the various taxa. B. Clarke and co-workers from Nottingham deal with genetic variation, showing how pulmonates are particularly favourable material for demonstrating the action of natural selection on genetically determined visible characters.

Convergence is widespread throughout the Mollusca, the diversely originating slugs being notable examples. Liable to become major pests, control, as P.J. Hunter stresses, often depends on knowledge of their ecology. This leads on to the chapter by D.S. Brown on pulmonates as intermediate hosts for digenetic trematodes. No-one is better qualified than W.D. Russell-Hunter to discuss the ecology of freshwater pulmonates, and so we proceed by way of an account of the evolution of gastropods in ancient lakes by K.J. Boss to the final chapter on the distribution and ecology of

the Stylommatophora by J. Peake, co-editor with Vera Fretter of these volumes.

Volume 2B entitled *Economic Malacology*, by A.R. Mead represents a continuation of its author's wonderful Giant African Snail (*Achatina fulica*) published in 1961. This supreme malacological saga tells of the spread by a surprising variety of agencies across Asia and then over the Pacific islands of this relatively immense high spired snail. Its supreme attempts to enter the United States, first by way of California and more recently by way of Florida, were only with difficulty repelled. This volume brings the story up to date and adds to the debt all malacologists owe to its author.

Enough one hopes has been written to reveal the range and high competence of these volumes and their importance to zoologists working on both pure and applied aspects of their subject.

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Peptide synthesis

The Peptides. Edited by Erhard Gross and J. Meienhofer. Pp.435. (Academic: New York and London, 1979.) \$39.50.

THE publication of *The Peptides* by Schröder and Lübke in 1965 was a landmark in the literature of peptide synthesis. The intervening period has seen the continued rapid growth of the subject, both in the development of new methodologies (including solid-phase synthesis) and in biological importance, yet no major survey in the English language has appeared. This entirely new *The Peptides* edited by Gross and Meienhofer is thus particularly welcome. The overall scope of the multi-volume and multi-authored work has been widened as indicated by its subtitle, *Analysis, Synthesis, Biology*; this first volume deals with major methods of peptide bond formation.

The opening chapter (by Gross and Meienhofer) presents a broad survey of the properties of the peptide bond. The coverage is wide, embracing structure, cleavage and synthesis, and is necessarily superficial in some instances. A case in point is the section on conformational analysis in solution where the single example quoted (oxytocin) is atypical and gives a misleading impression of the field as a whole. It would now be generally agreed that in the absence of special constraints, short linear peptides have rather random conformations in free aqueous solution. Conformational analysis under these circumstances is less useful.

The following chapter (by J.H. Jones) surveys all the main methods for peptide bond formation. Much of the information is covered in greater detail in subsequent chapters. The author has perhaps wisely avoided overmuch comment on the relative merits and applicability of the various methods, though his personal experiences have been distilled most valuably into a section on the choice of individual activated esters. This is a useful introductory chapter, especially for the newcomer to peptide synthesis.

The following four chapters provide detailed coverage of the major methods of synthesis. An authoritative account by Bodanszky surveys a wide range of activated ester derivatives concluding with a most useful series of fully referenced tables. The azide and mixed carbonic anhydride methods are surveyed by Meienhofer. Inclusion of significant experimental detail in these chapters gives an excellent 'feel' for the various techniques. The carbodiimide method is reviewed by Rich and Singh. The volume is completed by a comprehensive account of the racemisation problem by D.S. Kemp.

It is inevitable that *The Peptides* will be essential reading for all engaged in or contemplating peptide synthesis. It will supplement (but not replace) the monumental reference work edited by Wunsch in Volume 15 of *Houben-Weyl's Methoden der Organischen Chemie* (1974). Further volumes of Gross and Meienhofer's compilation are awaited with great interest.

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obituary

Sin-itiro Tomonaga, 1906-1979

SIN-ITIRO TOMONAGA, who shared the Nobel Prize for Physics in 1965, died on 8 July 1979 at the age of 73. His memory will be cherished for many years to come because of his witty personality and his sparkling ideas which contributed to the development of quantum electrodynamics in the post-war period.

Tomonaga was born in 1906 in Tokyo as a son of an eminent philosopher, and moved to Kyoto in 1913 with his family. In 1923 he entered the Third High School, and in 1926 he was admitted in Kyoto Imperial University where he specialized in physics. Through these years he became acquainted with Hideki Yukawa, another Nobel laureate, and they studied together. They remained lifelong friends.

It was in 1931, two years after Tomonaga graduated from the university, that he met Yoshio Nishina. Nishina, fresh from Bohr's Institute in Copenhagen, visited Kyoto to give a series of lectures on quantum mechanics. He brought back not only the celebrated Klein-Nishina formula but also the stimulating Copenhagen spirit to Japan and inspired in the young audience a fascination for the new frontier. This gave Tomonaga an excellent opportunity to enjoy Nishina's generous personality, so that the following year he decided to accept an invitation from Nishina to work at the Institute of Physical and Chemical Research in Tokyo as his research associate.

Nishina directed Tomonaga's interest to quantum electrodynamics, and they started to work together on this subject. This was the beginning of Tomonaga's life work, for which he was later awarded the Nobel Prize. Those stimulating days in the free atmosphere of the institute shaped a fertile brain that came into blossom a decade later. Tomonaga often recollected that the joint colloquia at the institute were a constant inspiration to him.

In 1937, he went to Leipzig to join the theoretical group led by Professor Werner Heisenberg and stayed there for two years. During his sojourn there he published a paper on thermal and mechanical properties of nuclear matter. In 1939, after his return home from Europe he was awarded the degree of Doctor of Science from Tokyo Imperial University upon presentation of his dissertation based on the outcome of his stay in Leipzig. In 1940, he married Ryoko Sekiguchi, a daughter of the then director of the Tokyo Astronomical Observatory. In 1941, he joined the



faculty of Tokyo Bunrika University which was later absorbed into the Tokyo University of Education in 1949.

At that time (1941) he concentrated his thoughts on a non-perturbative approximation method in meson theory which developed afterwards into the strong coupling theory, as well as on the generalization of Dirac's many-time formalism to field theory. In the same year World War II broke out, and everybody had to get through a very difficult time. However, Tomonaga's spirits were high in those days, and he was widening his repertory of specialties ranging from field theory to electronics. He completed a relativistically invariant formulation of the quantum theory of wave fields, the so-called super-multiple-time formulation, as the ultimate form of Dirac's many-time formalism. He had to wait for the war to end, however, before his theory could bear fruit: the renormalization programme. During the war he developed a theory of microwave systems as an application of the S-matrix formalism. He also clarified the oscillation mechanism of magnetrons on the basis of an intuitive model and secular perturbation. For these contributions he was awarded the Japan Academy Prize in 1948 together with his collaborator Masao Kotani.

When the war was over in 1945, Tokyo

had been reduced to ashes, and everybody had to go through all kinds of hardships. Research on nuclear physics was banned in Japan, and the cyclotron built by Nishina was dumped in the Bay of Tokyo. Yet Tomonaga reached the loftiest pinnacle of his scientific activities in this post-war period. His deep-rooted idea on the renormalization programme was ripe, and he immediately started to work towards completion of his theory with his young associates. They gathered to hold seminars in the midst of ruins. At that time Japanese physicists had almost no access to scientific journals from abroad, but Tomonaga learnt of the discovery of the Lamb shift and its interpretation by Bethe through the popular science column of an American weekly magazine. This hint was sufficient to convince him of the correctness of his idea and soon he established his version of quantum electrodynamics. Another fruit was the formation of the Tomonaga school through his collaboration with young physicists in this period.

In 1949, Tomonaga was invited to the Institute for Advanced Study in Princeton and stayed there for a year. There, he broke new ground in the many-body problem.

In 1951, Nishina died, and Tomonaga had to assume many of his administrative responsibilities. After reaching the highest scientific achievement, Tomonaga then paid special attention, in Nishina's place, to the improvement of the working conditions of young scientists. He dedicated his best efforts to the cause of starting research laboratories and facilities such as the Norikura Cosmic Ray Observatory and the rebuilding of a cyclotron at the Institute of Physical and Chemical Research. He also took the leadership in establishing the Institute for Nuclear Study and later the National Laboratory for High Energy Physics.

In 1956, he was elected President of the Tokyo University of Education and remained in this position until 1962. Then he served as President of the Science Council of Japan from 1963 to 1969. Since 1964 he was President of the Nishina Memorial Foundation established to encourage young atomic scientists. This was the position that he held until his death. In 1965, Tomonaga shared the Nobel Prize for Physics with Julian Schwinger and Richard P. Feynman. His scientific achievement was honoured also with the Order of Culture, Japan, in 1952 and the Lomonosov Medal, USSR, in 1964. He

was a member of a number of academies and learned societies both domestic and foreign.

Almost a year ago I visited him in a hospital and we talked about a book he was preparing, but then I had not the slightest idea that I was never to see him again.

Kazuhiko Nishijima

Gilbert Stead

PROFESSOR STEAD, who died on 5 July 1979, aged 91, was one of a small group of physicists who helped to establish radiology as an independent medical discipline.

Medical interest in X-rays was sparked off by a lecture given by Silvanus Thompson to the Clinical Society of London in March, 1896, and during the next 25 years, largely through the enthusiasm of younger doctors with no special training, the use of X-rays for diagnostic purposes spread rapidly. These pioneers often built and maintained their own apparatus!

It was not until the Cambridge Diploma in Medical Radiology and Electrology (D.M.R.E.) was instituted in 1919 that such training became available. A course in radiological physics was begun by J.A. Crowther at the Cavendish Laboratory in 1920, with Rutherford's active support; S. Russ and L.H. Clark provided similar courses in London. In 1924, Stead took over the Cambridge teaching as University Lecturer in Physics as Applied to Medical Radiology and he continued it until 1938. From 1927 until 1942, when this Diploma was discontinued, he was also Secretary to the Diploma Committee.

Stead's connection with Guy's Hospital began in 1923 when he was appointed Reader in Physics in the Medical School, a part-time post. The facilities available there only allowed for under-graduate teaching, but in 1938 he was elected by the University of London to a Chair of Physics and was given proper laboratory accommodation. Unfortunately, the outbreak of war in 1939 caused evacuation to Tunbridge Wells and it was not until 1945 that he could begin to build up his department as a centre for applying physics in medicine.

He set up courses for the newer radiological diplomas which had been instituted by the University of London and by the Royal Colleges of Physicians and of Surgeons; and he acted as examiner for them at various times until he retired in 1953, having contributed greatly to setting the standards of teaching and examination which made these diplomas universally accepted. He was President of the British Institute of Radiology (the original Röntgen Society, founded by a group of

medical men in 1897) in 1947/48 and delivered the Silvanus Thompson Memorial Lecture there in 1959. He was Honorary Consulting Physicist to Guy's Hospital and a Governor of the Medical School.

During his 30 years at Guy's Stead established a great reputation as a teacher. Some 3,000 undergraduates (who had to "do" physics in their first year, not always willingly!) attended his courses. Gentle in manner, patient and understanding their difficulties, he succeeded not only in overcoming their foibles but also in winning their affection. A text-book, *Elementary Physics*, which he wrote with such students in mind, is now (1979) in its eleventh edition; it was first published in 1923!

Stead outlived most of his contemporaries, but many younger people, not only in this country, remember his retiring, courteous personality. He was once described as "shrewd in discussion, wise in counsel and temperate in judgment." Those who had the privilege of working closely with him knew him also as a loyal friend.

C.B. Allsopp

Carl L. Hubbs

PROFESSOR CARL LEAVITT HUBBS, one of the world's most distinguished ichthyologists, doyen of that tribe in America, a true naturalist and a great personality, died on 7 July 1979 at the age of 84.

Born in Williams, Arizona, shortly after his mother was rescued by a train crew who had found her lost in the desert while driving a wagon, Carl Hubbs ended his distinguished career as Professor Emeritus of the Scripps Institute of Oceanography (University of California) where he had worked since 1944.

The early arousal of his interest in natural history he attributed to his maternal grandmother, one of the first woman physicians; it was further expanded by his father, a self-taught mineralogist, and was fully developed at Stanford University under the rigorous tutelage of that master ichthyologist David Starr Jordan. Hubbs obtained his bachelor's (1916) and master's (1917) degrees at Stanford, and his doctorate (1927) at the University of Michigan. After three years as a curator in the Field Museum, Chicago, he returned, for 24 years, to the University of Michigan. There, apart from his lecturing duties he was curator of vertebrates in the University Museum, and was responsible for building up that museum's collection of fishes, now one of the finest in the world.

Hubb's command of ichthyology was awesome, his research interests extensive

but penetrating; their results, published in over 600 papers, could never be criticised for being superficial or trivial. Perhaps the most characteristic feature of his taxonomic work was the way in which he took account of the animal's biology and viewed his subjects in their historical context. It is no wonder that Hubbs contributed so much to unravelling the historical biogeography of American freshwater fishes, nor that his interests should have turned to distributional problems in marine fishes as well. That, in turn, led him into oceanographical and meteorological research, while his freshwater studies took him into the fields of archaeology and human palaeoecology.

With his wife Laura, a mathematician and a dedicated companion, Carl Hubbs pioneered several studies into the genetics of fishes, in particular the application of hybridization studies to the systematics of several taxonomically "difficult" groups. Together they were responsible for discovering the now famous matroclinous and gynogenetic reproduction of the Amazon Molly, *Poecilia formosa*.

Problems of speciation and evolution, especially amongst the rich freshwater fish faunas of north and middle America, also attracted Hubbs. Interestingly, the phrase "new systematics" was first coined by him several years before it was used, apparently as a neologism, by Huxley as a title for that now classic collection of taxonomic essays he edited in 1940.

Conservation was a long standing concern of Hubbs'. He was instrumental in protecting forests, elk, fur seals, the Pacific gray whale and, after a forcefully conducted fight, the desert pupfish of Devil's Hole, Nevada.

That quite inadequate summary of Carl Hubbs' research and influence on World ichthyology has overlooked his role in the development of many American research institutes and professional societies, his importance as a teacher, especially of post-graduate students, and his dedicated involvement in the more pragmatic aspects of his great love, ichthyology.

Many honours were conferred on him, including election to the National Academy of Sciences, the award of the Joseph Leidy Medal, the Shinkishi Hatai Medal of Japan, the sole honorary membership of the Japanese Ichthyological Society, and election to Foreign Membership of the Linnean Society of London.

Informally Carl Hubbs will long be remembered with warm affection by those who knew him, in particular by fellow ichthyologists for whom, irrespective of their status in the discipline, and despite his many commitments, he could always find time to share his knowledge and give his critical encouragement. Historically his contributions to ichthyology will ensure him a degree of immortality vouchsafed to few.

P.H. Greenwood